NICOTINIC ACID AND THE LEVEL OF PROTEIN INTAKE IN THE NUTRITION OF THE PIG [1]

MAXWELL M. WINTROBE,[2] HAROLD J. STEIN, R. H. FOLLIS, JR. AND STEWART HUMPHREYS [2]

Departments of Medicine, Johns Hopkins University, Baltimore, and University of Utah, Salt Lake City

THREE FIGURES

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Shortly following discovery of the action of nicotinic acid in "black tongue" in dogs (Elvehjem et al., '38), Chick and co-workers ('38) reported the dramatic effect brought about by the administration of nicotinic acid to pigs which had been fed a diet consisting of white maize, peameal, purified casein, cod liver oil, and salt mixture. A need for nicotinic acid on the part of the pig was also observed by Hughes ('38), Madison, Miller and Keith ('39), and in our laboratory (Wintrobe, '39).

In all of the experiments cited above, the diets used were no doubt incomplete in several respects. Although the use of nicotinic acid produced a marked improvement in the growth and appearance of pigs receiving only thiamine and riboflavin, it was soon evident in our own studies that additional vitamins are required by these animals. Recently we showed (Wintrobe, Miller, Follis et al., '42) that excellent growth and development take place when supplements of pyridoxine, choline, and calcium pantothenate are furnished in addition to thiamine, riboflavin, and nicotinic acid. It was then that an attempt was made to find out what the effect in pigs is when, of the above six vitamins only nicotinic acid is omitted. To our surprise, no ill effects were observed.

The basal diet used by Chick et al. ('38) was relatively low in protein (about 14%) and probably lacked certain B vitamins other than nicotinic acid (Waisman and Elvehjem, '40). This was true also of the diet used by Hughes ('38). Similar deficiencies may have been present in

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[2] Present address, School of Medicine, University of Utah, Salt Lake City, Utah.

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the diet of Madison et al. ('39), although exact information is lacking. Our animals (Wintrobe, '39) were fed as much as 26.1% casein, but the diet was supplemented only with thiamine and riboflavin and was deficient at least in pyridoxine (Wintrobe, Follis, Miller et al., '43) and pantothenic acid (Wintrobe, Follis, Alcayaga et al., '43) as well as in nicotinic acid. It is of interest in this connection to note that the diet of the pellagrin is poor in protein and minerals as well as in vitamins and that the Goldberger experimental diet supplies protein which is poor qualitatively as well as quantitatively.

In view of these facts it seemed possible that, when of the known dietary factors required by the pig nicotinic acid alone was not furnished, our failure to note any ill effects might be explained by some alteration in the requirement for nicotinic acid under the conditions of our experiment. In particular it seemed possible that the high level of protein in the diet might be a factor of importance.

EXPERIMENTAL PROCEDURE

The animals described in this study consisted of two series. The more important one consists of seventeen pigs derived from five litters, all of which were received at the same time and were placed on experiment at approximately the same age (69 to 77 days of age). These pigs were divided into six experimental groups as evenly as possible, having in mind litter and weight. The remainder, which comprise forty-four pigs, are additional animals studied from time to time which furnish supplementary data concerning the role of nicotinic acid in the nutrition of the pig. These also include litter mates divided among various experimental groups. The total of sixty-one pigs make up nine groups of pigs and several subgroups, as shown in table 1.

The "high protein" diet is our standard basal diet, already described (Wintrobe, Miller, Follis et al., '42) of which 26.1% is "crude" casein, the remainder consisting of sucrose, lard, and a salt mixture. In the "low protein, high fat" diet, the lard was increased to make up for the reduction of casein, as follows: crude casein, 10.0%; sucrose, 56.8%; lard, 26.8%; and salt mixture, 6.4%. In the "low protein, low fat" diet, sucrose was increased to make up for the reduction of casein, as follows: crude casein, 10.0%; sucrose, 73.8%; lard, 11.0%; and salt mixture, 5.2%. The diets were fed in such quantities that the caloric intake for all the animals was the same.

The Goldberger diet consisted of ground white maize (76.4%), peameal (10.3%), crude casein (6.4%), cod liver oil (4.4%), and salt mix-

ture (2.5%). The protein content of this mixture was approximately 17.3%.

All diets were supplemented with cod liver oil or blended fish oils,[3] 0.5 gm. per kilogram body weight daily, as in experiments previously described (Wintrobe, Miller, Follis et al., '42).

TABLE 1

Average daily weight gain of pigs fed various experimental diets.

GROUP NO.	BASAL DIET				CRYSTALLINE VITAMIN SUPPLEMENTS[1]	NO. OF PIGS	AGE EXPERIMENT		AVERAGE DAILY WEIGHT GAIN
	Casein	Fat	Carbohydrate	Type of casein			Begun on days – to –	Ended on days – to –	
	%	%	%						gm.
1	26.1	11.0	57.7	"Crude"	T	5	67–123	119–247	107
2	26.1	11.0	57.7	"Crude"	TR	4	67– 80	106–237	108
						2	16– 22	78–122	67
						2	34	99–128	42
3	26.1	11.0	57.7	"Crude"	TRN	11	64–107	87–300	123
						2	16– 22	72– 74	58
4	26.1	11.0	57.7	"Crude"	TR ChP	3	70– 77	118–238	154
5	26.1	11.0	57.7	"Crude"	TR B_6ChP	6	70– 90	210–292	413
5a	26.1	11.0	57.7	Vitamin-free[2]	TR B_6ChPIp	2	16– 22	136–225	360
6	26.1	11.0	57.7	"Crude"	TRNB_6ChP	9	70–106	216–448	336
6a	26.1	11.0	57.7	"Crude"	TRNB_6ChPIp	1	34	213	433
6b	26.1	11.0	57.7	Vitamin-free	TRNB_6ChP	3	87– 88	236–238	281
7	10.0	26.8	56.8	"Crude"	TR B_6ChP	3	69– 77	134–227	51
7a	10.0	11.0	73.8	"Crude"	TR B_6ChPIp	2	21– 22	65–134	51
8	10.0	26.8	56.8	"Crude"	TRNB_6ChP	3	69– 77	95–225	162
9	17.3	7.4	75.3	"Crude" (Goldberger)[3]	TR B_6ChP	3	71– 77	114–230	167

[1] T refers to thiamine, 0.25 to 0.52 mg. per kg. body weight daily; R, riboflavin, 0.12 mg.; N, nicotinic acid, 1.20 mg.; Ch, choline chloride, 10.0 mg.; P, calcium pantothenate, 0.50 mg.; I, inositol, 1.20 mg.; p, para-aminobenzoic acid, 0.50 mg.

[2] From SMA Corporation, Cleveland, Ohio.

[3] "Goldberger" diet: ground white maize, 29.8 gm.; peameal, 4.0; crude casein, 2.5; cod liver oil, 1.7; salt mixture, 1.0 gm. Protein consists of casein, 6.5%; vegetable, 10.8%.

Complete details concerning the management of the animals have been published elsewhere (Wintrobe, Miller, Follis et al., '42; Wintrobe, Miller and Lisco, '40). The data presented in this report have been gathered since 1937 (Wintrobe, Mitchell and Kolb, '38). Throughout this period, the source of the animals, their handling, and their diet, even including the source of the casein fed, were not changed. The only change that was made was in the mineral mixture. The one first used (Wintrobe, Mitchell and Kolb, '38) was modified in June, 1939, to in-

[3] Mead Johnson and Company, Evansville, Ind.

clude copper, manganese, zinc, and cobalt, and ferric pyrophosphate replaced ferrous sulphate (Wintrobe, Miller and Lisco, '40).

The pigs were kept in specially constructed metabolism cages designed to minimize contact of urine with feces.

The procedure in these studies was to feed pigs the basal ("high protein") diet when they were received (about 21 days of age) together with 3 gm. of yeast per kilogram body weight daily. When the experimental period was commenced, the only source of the B complex, aside from the minimal amounts possibly present in the basal diet, was in the form of the crystalline vitamin supplements specified for each group.

In comparing the various animals, attention should be paid to two important factors, namely, litter mates and the time when an experiment was begun. The latter factor is important since older animals had a better opportunity to store a supply of all the nutritive factors present in yeast than those started on a mixture of crystalline vitamins at a very early age.

From this viewpoint the observations on the following seventeen pigs, referred to above, are to be considered particularly significant: Litter XXX, pigs 6-87, 7-02 and 6-81 (groups 4, 8 and 9); litter XXXI, pigs 6-84, 6-93 and 6-99 (groups 5, 6 and 7); litter XXXII, pigs 6-88, 6-85, 7-00, 7-04, 7-06, 6-82 and 6-83 (groups 4, 5, 7, 8 and 9); litter XXXIV, pigs 6-89 and 6-95 (groups 4 and 6); litter XXXV, pigs 6-86 and 7-01 (groups 5 and 7). Also worthy of special attention are the pigs of the following litters which are to be found in a variety of experimental groups: Litter XXVI, pigs 6-55, 6-51, 6-59, 6-57 (groups 3, 5, 6, 6b); litter XXVII, pigs 6-68, 6-63, 6-65, 6-61 (groups 3, 5, 6, 6b); litter XXVIII, pigs 6-69, 6-72, 6-75, 6-78 (groups 3, 5, 6, 6b); litter XLIII, pigs 7-67, 7-69, 7-73, 7-71 (groups 2, 3, 5a, 7a); litter XLIX, pigs 7-68, 7-70, 7-74 (groups 2, 3, 5a).

RESULTS

The role of nicotinic acid. The data for growth are summarized in table 1 and the growth curves for the individual pigs are shown in figure 1. Comparison of groups 1 and 2 with the eleven pigs of group 3 which received crystalline vitamin supplements and no yeast at a correspondingly late age (64 to 107 days), reveals a somewhat better growth rate for the animals receiving nicotinic acid in addition to thiamine and riboflavin, as compared with those receiving only the last two vitamins. The animals receiving nicotinic acid not only were larger than the pigs not receiving this vitamin (fig. 2) but their coats were in better condition, their muscles were less flabby, and there was less diarrhea. These

pigs, however, were not litter mates. Two pigs (7-69, 7-70) were fed no yeast when they were received, but were given thiamine, riboflavin, and nicotinic acid at once (16 to 22 days of age). These animals grew very poorly, and no better than their litter mates who were given only thiamine and riboflavin at the same age (7-67, 7-68).

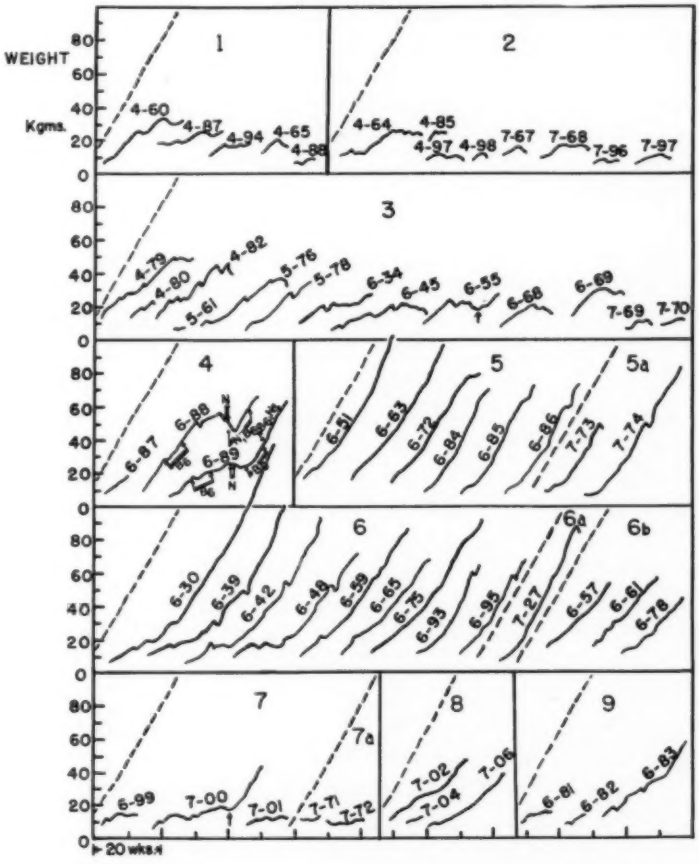

Fig. 1 Growth curves of pigs in various experimental groups. Note that the growth of pigs fed a high protein diet and receiving no nicotinic acid (group 5) was as good as that of pigs fed this vitamin (group 6) even when pigs given vitamin-free casein are compared (groups 5a and 6b). However, when the basal diet was low in protein, the growth of pigs given no nicotinic acid (groups 7 and 7a) was not as good as that of pigs receiving this vitamin (group 8).

Arrows indicate restoration of normal (high protein) diet and feeding of all necessary vitamin supplements to pigs 6-55 (group 3) and 7-00 (group 7). Pigs 6-88 and 6-89 (group 4) were given, from time to time, pyridoxine (B_6) and nicotinic acid (N).

The pigs mentioned above as developing relatively well when the experimental deficiency was commenced at 2 or 3 months of age instead of at 3 weeks, failed eventually also. Their growth became impaired, diarrhea persisted, anemia, epileptiform convulsions, and marked ataxia together with extensive degenerative changes in the nervous system developed. We have been able to show that these symptoms resulted from deficiencies of pyridoxine (Wintrobe, Follis, Miller et al., '43) and of pantothenic acid (Wintrobe, Follis, Alcayaga et al., '43).

When pyridoxine, calcium pantothenate, and choline were furnished in addition to thiamine, riboflavin, and nicotinic acid, animals receiving the same basal diet as those in groups 1, 2 and 3 grew very well, and no pathological changes occurred (group 6). The addition of inositol and p-aminobenzoic acid to the group of six vitamins mentioned above was associated with better growth in one animal (group 6a) as compared with group 6 as a whole. This difference, however, may not be significant for three of the nine pigs in group 6 (6-30, 6-93, 6-95) grew almost as well as this pig (average daily weight gain, 400, 396 and 394 gm., respectively). Good, though somewhat less satisfactory growth occurred in three pigs fed vitamin-free (group 6b) instead of crude casein (group 6) in association with the six "B" vitamins (compare litter mates 6-59 and 6-57, 6-65 and 6-61, 6-75 and 6-78).

Comparable animals given the same basal diet and the same vitamin supplements as those mentioned above (group 6) except that no nicotinic acid was supplied (group 5) showed no signs of nutritional deficiency except for slightly less satisfactory growth in certain instances as compared with those given this vitamin, and at autopsy no pathological changes attributable to deficiency were found. In table 2 data for comparable animals are presented and weight gain, expressed in terms of percentage of initial weight, is shown.

Neurological changes were absent. In none of the pigs of groups 5, 5a, 6, 6a or 6b did anemia or other morphological changes in the blood develop. When pyridoxine also was omitted (group 4), the changes which occurred (impaired growth, microcytic anemia, epileptiform convulsions, sensory neuron degeneration) were the same as those which develop in pyridoxine deficiency alone (Wintrobe, Follis, Miller et al., '43).

It is difficult to determine from our data whether "crude" casein supplied an unknown factor making it possible for pigs to dispense with dietary nicotinic acid. The pigs of group 5a grew well, even though they received vitamin-free casein. Nevertheless a direct comparison with the pigs of group 5 is not possible since groups 5 and 5a did not

contain litter mates. Furthermore, the two pigs of group 5a were given inositol and p-aminobenzoic acid in addition to the five vitamins furnished group 5, and this may or may not have contributed to the excellent growth of the pigs in group 5a.

From the standpoint of storage of nicotinic acid as a factor in influencing the need for dietary nicotinic acid, it is to be noted that the animals in group 5a received the crystalline vitamin supplements at a very early age (16 to 22 days) without opportunity to store growth factors available in yeast. In this respect they were at a disadvantage as compared with the pigs of group 5, for storage might have occurred in the case of the latter pigs. In any event, the excellent growth of the pigs

TABLE 2

Comparison of growth in individual animals.

GROUP 5 "High protein, no nicotinic acid"			GROUP 6 "High protein with nicotinic acid"		
Pig no.	Initial weight	Weight gain	Pig no.	Initial weight	Weight gain
	kg.	%		*kg.*	%
6-51	16.0	657	6-59	9.0	978
6-63	14.5	675	6-65	10.1	673
6-72	13.2	606	6-75	11.1	840
6-84	11.7	570	6-93	10.9	608
6-85	11.6	640	6-95	10.4	648
6-86	11.0	654			
GROUP 7 "Low protein, high fat, no nicotinic acid"			GROUP 8 "Low protein, high fat, with nicotinic acid"		
6-99	11.6	128	7-02	15.8	296
7-00	10.4	179	7-06	8.6	459
7-01	10.5	127	7-04 [1]	10.2	116

[1] Died after only 26 days on experiment.

given vitamin-free casein but no nicotinic acid (group 5a) makes it clear that under the experimental conditions described, the pig can grow well in the absence of nicotinic acid.

The role of protein. When animals receiving the "low protein" diet were given all of the vitamins already mentioned with the exception of nicotinic acid (groups 7 and 7a), growth was extremely poor (fig. 3). The coats of the pigs were rough and untidy. Diarrhea and poor appetite developed in all three pigs of group 7 ("low protein, high fat, no nicotinic acid"), and severe normocytic anemia also occurred, the red cell counts being 1.88, 2.33, and 3.54 millions, respectively, per cubic millimeter. Furthermore, although no definite abnormality in gait was observed during life, slight degenerative changes were found in the

posterior root ganglion cells of two of the three animals in group 7 and rather extensive chromatolysis in the dorsal root ganglia was present in the third. Only one of the pigs of group 7a ("low protein, low fat, no nicotinic acid") developed severe diarrhea. There was little anemia in either of these pigs. In the nervous systems of these two pigs, rather

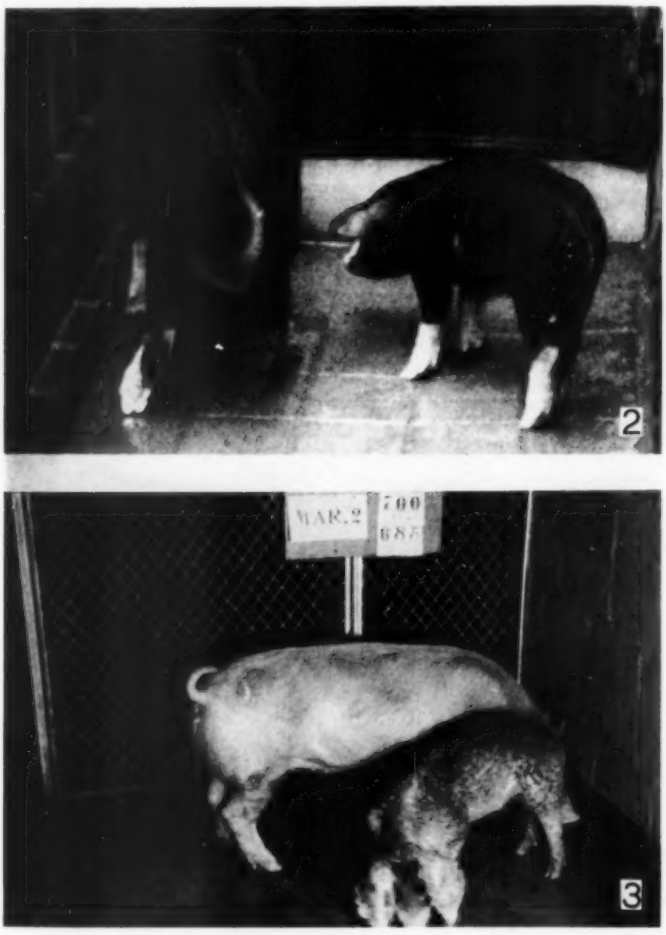

Fig. 2 Comparison of a pig of group 2 (4-98) with one of group 3 (4-82). The larger of the two pigs received nicotinic acid in addition to thiamine and riboflavin as supplements to the high protein basal diet. The smaller pig was given no nicotinic acid.

Fig. 3 Comparison of a pig of group 5 (6-85) with one of group 7 (7-00). Neither of these pigs was given nicotinic acid, but the smaller one was fed a diet low in protein. These pigs were litter mates.

extensive chromatolysis was found in the dorsal root ganglion cells of one (7-71) but there was no definite myelin degeneration. In the second pig, 7-72, the nervous tissues showed no lesions. It is interesting to note that the degenerative changes when present were found in the small ganglion cells. At autopsy extensive infections were found in the pigs of group 7, but not in group 7a. The cause of death in the two animals of the latter group was not clear.

Pigs fed a low protein, high fat diet, but furnished nicotinic acid (group 8) looked much better nourished than the pigs described above, even though their growth was impaired as compared with that of pigs given a greater proportion of protein (group 6). The growth curves of litter mates 7-00 and 7-06 of groups 7 and 8 may be compared with one another. Pigs 6-99, 7-02 and 7-01 came from other litters but were studied at the same time and under the same conditions as pigs 7-00 and 7-06. The coats of the animals in group 8 were clean, their hair was in good condition, no diarrhea developed, and only very slight normocytic anemia occurred (R.B.C. 5.90, 6.50 and 5.40, respectively, as compared with values of 6.50 million or higher in the pigs of groups 6). No pathological changes in the nervous system or other tissues were found at autopsy. Weight gain comparisons for the individual animals are presented in table 2.

One pig in group 7 (pig 7-00) was given 100 mg. nicotinic acid daily. The diarrhea ceased, the appearance of the pig improved, and its weight increased. The anemia was not altered. When the low protein diet was changed to the high protein type, further weight gain occurred, but the anemia persisted. It was not possible to influence the anemia appreciably even by the administration of liver extract or desiccated whole liver. When this animal was killed, severe infection was found (multiple abscesses: subcutaneous, lung, liver, spleen, peritoneum). It is possible that infection may have been at least in part responsible for the anemia noted in group 7, and it is likely that infection interfered with a hematopoietic response which might have occurred in pig 7-00. Therapy was attempted only in one other instance, pig 7-01 of group 7. Casein, 10 gm. per kilogram body weight daily, was fed in addition to the regular diet. No benefit was observed, but here again infection (lobular pneumonia) was found when the pig died 23 days later.

Two of the three pigs given the Goldberger ration supplemented with B vitamins except nicotinic acid (group 9) died early with widespread infection. Both had severe normocytic anemia (R.B.C. 2.69, 3.80 million, respectively). Their growth had been poor (103 and 130 gm. average daily weight gain, respectively), but their gait was normal. Extensive

degenerative changes were found in the posterior root ganglion cells, but the changes were confined to the small cells. One pig (6-83) survived a long time, grew moderately well, and had only slight anemia (R.B.C. 5.70 million). The better growth of this animal (230 gm. average daily weight gain) as compared with that of a litter mate (7-00) given no nicotinic acid and a low protein diet (group 7) may have been due to the higher content of protein in the Goldberger diet (6.5% casein, 10.8% vegetable protein, as compared with 10% casein). Pathological changes were found in the spinal ganglia in this animal, as in its mates, but the changes were not extensive. Lobular pneumonia was also present at autopsy.

Chemical studies. In an attempt to assess the degree of nicotinic acid deficiency in the different experimental groups, measurements of the urinary nicotinic acid, nicotinuric acid, trigonelline, and related compounds were carried out. The cyanogen bromide-p-aminoacetophenone method (Harris and Raymond, '39) was adapted for use with the Evelyn photoelectric colorimeter using a 440 filter. Each sample of urine was subjected first to three types of hydrolysis, according to the method of Melnick, Robinson and Field ('40). Cyanogen bromide was omitted from the blank since in our experience this procedure gave the most consistent values. The value for the sample subjected to ½ hour of acid (4N HCl) hydrolysis is thought to include all of the excreted nicotinic acid, nicotinamide, free pyridine, nicotine, approximately two-thirds of the nicotinuric acid, and any other unknown pyridine compounds that react with the reagents. The values obtained following 5-hour acid hydrolysis include, in addition to the above, all of the nicotinuric acid. Following alkaline (9N NaOH) hydrolysis of the urine for ½ hour, the value obtained is regarded as including all of the pyridine compounds determined by use of the prolonged acid hydrolysis plus about one-third of the voided trigonelline. By using conversion factors, it is possible to determine the total amount of trigonelline excreted; however, since these values are only semiquantitative, we have recorded only the actual values obtained through the chemical analyses. These data are summarized in table 3.

The urine samples were collected in two 24-hour periods and the sample for each period was analysed separately. All urine samples were collected under toluene. The tabulated results represent the average for the 2 days. The range of variation is also indicated. It is recognized that where the range of values is so wide, the average has little meaning. Averages have been recorded only to give some concept of the trend.

TABLE 3

Urinary excretion of nicotinic acid derivatives by pigs fed various experimental diets. All data are in milligrams per 24 hours (average and range).

	DIET AND SUPPLEMENTS						
	High protein plus				Low protein plus		Goldberger
HYDROLYSIS	TRN — 3[1] (10)[2]	TRChP — 4 (14)	TRB_3ChP — 5 (45)	$TRNB_3ChP$ — 6 (22)	TRB_3ChP — 7 (12)	$TRNB_3ChP$ — 8 (11)	TRB_3ChP — 9 (8)
½ hr. Acid (4 N HCl): "Nicotinic acid"	5.32 2.37– 7.40	1.87 0.–3.83	3.04 0.–11.96	3.82 0.56– 8.24	0.63 0.–3.18	1.63 0.– 6.85	1.56 0.– 7.14
5 hr. Acid (4 N HCl): "Nicotinuric acid"	5.23 2.36– 7.61	2.81 0.–5.40	4.15 0.–20.31	5.95 0.40–28.34	0.71 0.–2.35	2.43 0.– 7.17	3.37 0.–15.75
Alkaline (9 N NaOH): "Trigonelline"	6.06 1.35–12.19	2.65 0.–7.98	4.08 0.–22.92	4.75 0.57–16.27	1.08 0.–2.64	3.18 0.62–10.68	3.53 0.–12.70

[1] For meanings of these symbols see footnotes to table 1. The number signifies the number of the group.

[2] Numbers in parentheses indicate number of determinations.

In dogs fed a diet high in nicotinic acid, Sarett ('42) reported "nicotinic acid" urinary excretion values of 0.4 to 1.6 mg. and "trigonelline" of 1.5 to 7.3 mg. per 24 hours. In a dog during the onset of black tongue, the "nicotinic acid" was within the same range, but the "trigonelline" fell as low as 1.9 to 0.1 mg. Measurements of urinary "nicotinic acid" values in human subjects, both normal and deficient, have varied over a very wide range (Field et al., '41). In deficient subjects, Field and his associates ('41) noted an average, but not a constant, decrease in the urinary excretion of substances reacting like nicotinamide and a more marked and consistent decrease in the urinary excretion of trigonelline in the deficient subjects.

In our various experimental groups, there was a similar extremely wide range of variation in the excretion of nicotinic acid derivatives. One fact stands out, however. In the pigs fed the low protein diet without a supplement of nicotinic acid (group 7), (the only animals which showed marked evidence of nutritional deficiency somewhat resembling that ascribed to a lack of nicotinic acid), the excretion of nicotinic acid derivatives was very low and remained at a low level. Both the average and the maximum levels in this group were substantially lower than in any other experimental group. It is also possibly significant that, of all the groups of pigs receiving supplements of nicotinic acid, only those fed a low protein diet (group 8) at times excreted no "nicotinic" or "nicotinuric acid."

It is of interest that, in general, substantially lower values for the excretion of "trigonelline" were observed in all groups not receiving nicotinic acid as a supplement. The highest maximal values for "nicotinic acid" and "nicotinuric acid," however, were found in the pigs fed a high protein diet without nicotinic acid supplement (group 5) while the maximal "trigonelline" value in this group was only exceeded by that found in group 6 which was fed the same diet and received a supplement of nicotinic acid.

Najjar and Holt ('41) described the presence of certain fluorescent substances in the urine which they designated as F_1 and F_2. The latter is found in normal urine and tends to disappear in the presence of nicotinic acid deficiency. This substance (F_2) has been identified as N-methylnicotinamide (Huff and Perlzweig, '43). Subsequently Sarett ('43) showed that the so-called urinary "trigonelline" fraction is composed chiefly of N-methylnicotinamide although other methylated nicotinic acid derivatives are also included. Measurements of F_2 (N-methylnicotinamide) were made in a number of our pigs. The presence of this substance paralleled rather closely the excretion of the methylated

nicotinic acid derivatives ("trigonelline" fraction). In those animals receiving no nicotinic acid supplement, F_2 excretion was markedly diminished.[4]

DISCUSSION

Under the conditions of these experiments good growth and development occurred in pigs when they were given a diet containing a high percentage of a good grade of protein, supplemented with thiamine, riboflavin, pyridoxine, choline, and pantothenic acid, as well as vitamins A and D and sufficient quantities of fat, carbohydrate and minerals. This diet did not furnish significant quantities of nicotinic acid, even when "crude" casein was used. Microbiological assay (Snell and Wright, '41) of the casein used in most of our experiments showed it to contain less than 0.6 μg. nicotinic acid per gram.[5] Since our animals received at most 9.5 gm. casein per kilogram body weight, no more than 5.7 μg. of nicotinic acid per kilogram was derivable from this source. As a further check, however, two pigs were fed vitamin-free casein. These pigs also developed well. It is to be noted that these last two pigs received an experimental diet lacking in nicotinic acid from the ages of 16 and 22 days, respectively. Significant post-natal storage of nicotinic acid therefore, can scarcely be a factor.

The importance of nicotinic acid in nutrition was first demonstrated through the use of diets deficient in more than one respect. It was shown recently, however, that in the case of the dog loss of weight, inflammation of the gums, and reddening of the palate occur even when a highly purified diet similar to that used in our experiments and supplemented with the same five "B" vitamins is furnished (Schaefer et al., '42). Somewhat similar observations have been made in the chick (Briggs et al., '43). Experiments in monkeys (Harris, '38) and, as stated earlier, in pigs, were carried out by the use of diets of the Goldberger type and, therefore, cannot be regarded as having demonstrated a need for nicotinic acid as a supplement to a diet which is otherwise complete. A dietary source of nicotinic acid is not required by the rat (Dann, '41) or the sheep (Pearson et al., '40). Wooley and Sebrell ('45) have recently presented evidence that nicotinic acid is an essential growth factor for rabbits fed a purified diet.

Powick,[6] in studies which have been in progress for several years at the Beltsville Research Center of the U. S. Department of Agriculture,

[4] We are indebted to Dr. V. A. Najjar for these determinations.

[5] We are indebted to Dr. Daniel Melnick of the Food Research Laboratories, Inc., for this assay.

[6] Powick, W. C., personal communication.

has found impaired growth in a high proportion of young pigs fed a synthetic ration containing large amounts of acid-washed, alcohol-extracted casein (25%) and supplemented with thiamine, riboflavin, pyridoxine and pantothenic acid but devoid of nicotinic acid. It is of interest, in regard to the discrepancy between our observations and those of Powick, that Handler ('43), using a purified diet like that which Schaefer et al. ('42) used successfully in producing nicotinic acid deficiencies, could not induce a deficiency state in dogs which completely resembled that of black tongue dogs on Goldberger diets. Furthermore the weight loss, anorexia and other symptoms which did develop, disappeared spontaneously. Handler remarked that the inconsistent behavior of dogs on a purified ration made it difficult to evaluate the nature of the physiological defects in nicotinic acid deficiency.

Such observations, as well as our own, give ground for the belief that other factors as well as nicotinic acid play a role in the development of the deficiency syndrome which has been attributed to lack of this vitamin alone. According to the studies reported here, protein is one such factor. Only when the diet of our animals contained a small percentage of protein and lacked nicotinic acid as well (groups 7 and 7a), did signs of nutritional deficiency appear. These included marked impairment of growth, diarrhea, poor appetite, a rough coat, and anemia. The importance of the combined deficiency of protein and of nicotinic acid is indicated by the comparatively good condition of animals given diets deficient only in protein (group 8) or in nicotinic acid (group 5).[7]

The possibility that a deficiency of certain essential amino acids might be a factor in the etiology of pellagra was postulated as long ago as 1914 by Voegtlin ('19–'20). At that time, Goldberger and his coworkers ('15) showed that the development of the disorder could be prevented when the diet was supplemented with milk, fresh meat, eggs, and dried beans. The classical diet which has been used in the experimental production of canine black tongue is poor in protein as well as in B vitamins.

It is recognized that the pyridine nucleotides play a role in protein metabolism (Ball, '39). Handler and Dann ('42) reported that nicotinamide inhibits the growth of rats, probably due to the deprivation of the animal's supply of methyl groups because of trigonelline synthesis. The inhibition of growth could be prevented by the administration of

[7] The view here expressed that the quantity of protein and perhaps, in particular, of certain essential amino acids in the diet, is important as affecting nicotinic acid requirement, finds further support in the recent observation by Krehl, Tepley, Sarma and Elvehjem ('45, Science, vol. 101, p. 489). These workers present evidence "that protein, or tryptophane, particularly, may have a profound effect on the nicotinic acid requirement."

methionine and by choline plus homocystine. Sarett, Klein and Perlzweig ('42) noted a decreased urinary excretion of nicotinic acid in dogs when the protein content of the diet was increased, and an increased excretion when the protein content was lowered. Their observation in the dog agrees with ours in the pig only in that an inter-relationship of protein and nicotinic acid is suggested; somewhat at variance is our finding that the excretion of nicotinic acid derivatives was lowest in pigs receiving a low protein diet not supplemented with nicotinic acid. The experiment of Sarett, Klein and Perlzweig did not include a group fed a diet deficient in both protein and nicotinic acid. Sarett and Perlzweig ('43) later reported that the nicotinic acid content of the livers of rats fed a low protein diet was low as compared with the livers of those fed high protein diets, whether the vitamin intake was high or low. Differences in species and in experimental methods make further comparisons of their observations and ours impossible.

None of these observations regarding the relationship of protein and nicotinic acid, however, serve to explain the dramatic improvement noted in two pigs by Chick and her co-workers ('38) when only nicotinic acid was added to the "Goldberger maize diet" which her animals were being fed. Nor do they explain the observations of other investigators (Hughes, '38; Madison et al., '39; Wintrobe, '39) which led to the conclusion that a dietary source of nicotinic acid is needed by the pig. Since the diets furnished in the above experiments were probably lacking in other B vitamins besides nicotinic acid, our findings would seem to indicate that in some way the pig is able to make up for a lack of nicotinic acid in the diet when thiamine, riboflavin, pyridoxine, choline, and pantothenic acid are furnished. Perhaps the presence of these vitamins is beneficial by making possible the bacterial synthesis of nicotinic acid in the intestines, or by synthesis of the vitamin in the body. Our attempt to show that synthesis of nicotinic acid was mediated in some way by pyridoxine, which was the reason for our group 4, gave inconclusive results. It is to be noted that nicotinic acid and pantothenic acid are closely related both in chemical properties and in distribution (Elvehjem, '40). Study of the biological interrelationship of these two vitamins would seem to be indicated. We have described elsewhere (Wintrobe, Follis, Alcayaga et al., '43) the severe colitis which develops in pigs deficient in pantothenic acid and have pointed out the similarity of these changes in the bowel to those which have been described under such titles as "necrotic enteritis" and "pig pellagra," and have been attributed to nicotinic acid deficiency.

Various investigators (Miller and Rhoads, '35; György, '38; Day et al., '40; Handler and Featherston, '40) have described anemia and leukopenia in various animals receiving black tongue-producing diets or diets lacking nicotinic acid. In our animals leukopenia was never noted. When nicotinic acid alone was lacking, no anemia or other morphological abnormalities were found, but when the diet was also poor in protein, severe normocytic anemia developed. Since severe infections occurred in these animals, no conclusions can be drawn regarding the significance of this anemia.

The measurement of the urinary excretion of nicotinic acid derivatives has not proved to be a wholly satisfactory method for assessing nicotinic acid deficiency (Perlzweig et al., '42). Nevertheless, it is of interest that a correlation between such excretion and the presence of signs of nutritional deficiency was observed in the pigs fed the low protein diet without nicotinic acid supplement.

The development of chromatolysis in the dorsal root ganglion cells of four out of five of the pigs receiving the low protein-nicotinic acid deficient diet (groups 7 and 7a) is interesting but its significance is not entirely clear.

SUMMARY

1. Observations in sixty-one pigs are described in which the effects of low and high protein diets supplemented with thiamine, riboflavin, pyridoxine, choline, pantothenic acid, inositol, and p-aminobenzoic acid, with and without nicotinic acid, are compared.

2. Young pigs fed a high protein (26.1% casein) diet supplemented with the first five vitamins named above, and not furnished with nicotinic acid, showed no signs of nutritional deficiency except for slightly less satisfactory growth in certain instances.

3. When the protein content of the diet was low (10% casein) the omission of nicotinic acid from the diet was associated with the development of signs of nutritional deficiency (markedly impaired growth, rough coats, diarrhea, poor appetite and severe anemia).

4. Only in the last mentioned pigs was there a marked and consistent reduction in the urinary excretion of nicotinic acid derivatives.

5. There appears to be a close nutritional relationship between protein and nicotinic acid.

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LITERATURE CITED

BALL, E. G. 1939 Chemical reactions of nicotinic acid amide in vivo. Bull. Johns Hopkins Hosp., vol. 65, p. 253.

BRIGGS, G. M., JR., T. D. LUCKEY, L. J. TEPLY, C. A. ELVEHJEM AND E. B. HART 1943 Studies on nicotinic acid deficiency in the chick. J. Biol. Chem., vol. 148, p. 517.

CHICK, HARRIETTE, T. F. MACREA, A. J. P. MARTIN AND C. J. MARTIN 1938 III. Curative action of nicotinic acid on pigs suffering from the effects of a diet consisting largely of maize. Biochem. J., vol. 32, p. 10.

DANN, W. J. 1941 The synthesis of nicotinic acid by the rat. J. Biol. Chem., vol. 141, p. 803.

DAY, P. L., W. C. LANGSTON, W. J. DARBY, J. G. WAHLIN AND V. MIMS 1940 Nutritional cytopenia in monkeys receiving the Goldberger diet. J. Exp. Med., vol. 72, p. 463.

ELVEHJEM, C. A. 1940 Relation of nicotinic acid to pellagra. Physiol. Rev., vol. 20, p. 249.

ELVEHJEM, C. A., R. J. MADDEN, F. M. STRONG AND D. W. WOLLEY 1938 The isolation and identification of the anti-black tongue factor. J. Biol. Chem., vol. 123, p. 137.

FIELD, H., JR., D. MELNICK, W. D. ROBINSON AND C. F. WILKINSON, JR. 1941 Studies on the chemical diagnosis of pellagra (nicotinic acid deficiency). J. Clin. Inv., vol. 20, p. 379.

GOLDBERGER, J., C. H. WARING AND D. G. WILLETS 1915 The prevention of pellagra. A test of diet among institutional inmates. Pub. Health Rep., vol. 30, p. 3117.

GYÖRGY, P. 1938 Nicotinic acid and prevention of nutritional panmyelophthisis in rats. Proc. Soc. Exp. Biol. Med., vol. 37, p. 732.

HANDLER, P. 1943 Use of highly purified rations in the study of nicotinic acid deficiency. Proc. Soc. Exp. Biol. Med., vol. 52, p. 263.

HANDLER, P., AND W. J. DANN 1942 The inhibition of rat growth by nicotinamide. J. Biol. Chem., vol. 146, p. 357.

HANDLER, P., AND W. P. FEATHERSTON 1943 The biochemical defect in nicotinic acid deficiency. II. On the nature of the anemia. J. Biol. Chem., vol. 151, p. 395.

HARRIS, L. J. 1938 The vitamin B_2 complex. VII. Further notes on "monkey pellagra" and its cure with nicotinic acid. Biochem. J., vol. 32, p. 1479.

HARRIS, L. J., AND W. D. RAYMOND 1939 Assessment of the level of nutrition. A method for the estimation of nicotinic acid in urine. Biochem. J., vol. 33, p. 2037.

HUFF, JESSE W., AND W. A. PERLZWEIG 1943 N-methylnicotinamide, a metabolite of nicotinic acid in urine. J. Biol. Chem., vol. 150, p. 395.

HUGHES, E. H. 1938 The vitamin B-complex as related to growth and metabolism in the pig. Hilgardia, vol. 11, p. 10.

MADISON, L. C., R. C. MILLER AND T. B. KEITH 1939 Nicotinic acid in swine nutrition. Science, vol. 89, p. 490.

MELNICK, D., W. D. ROBINSON AND H. FIELD, JR. 1940 Influence of the excretion of other pyridine compounds upon the interpretation of the urinary nicotinic acid values. J. Biol. Chem., vol. 136, p. 131.

MILLER, D. K., AND C. P. RHOADS 1935 Experimental production of loss of hematopoietic elements of gastric secretion and of liver in swine with achlorhydria and anemia. J. Clin. Inv., vol. 14, p. 153.

NAJJAR, V. A., AND L. E. HOLT, JR. 1941 The excretion of specific fluorescent substances in the urine in pellagra. Science, vol. 93, p. 20.

PEARSON, R. B., A. H. WINEGAR AND H. SCHMIDT 1940 Studies on the metabolism of nicotinic acid in the sheep. J. Nutrition, vol. 20, p. 551.

PERLZWEIG, W. A., H. P. SARETT AND L. H. MARGOLIS 1942 Studies in nicotinic acid metabolism. V. A test for nicotinic acid deficiency in man. J. Am. Med. Assn., vol. 118, p. 28.

SARETT, H. P. 1942 Studies in nicotinic acid metabolism. II. The fate of nicotinic acid in normal and black tongue dogs. J. Nutrition, vol. 23, p. 35.

———— 1943 A direct method for the determination of N-methyl derivatives of nicotinic acid in urine. J. Biol. Chem., vol. 150, p. 159.

SARETT, H. P., J. R. KLEIN AND W. A. PERLZWEIG 1942 The effect of the level of protein intake upon the urinary excretion of riboflavin and nicotinic acid in dogs and rats. J. Nutrition, vol. 24, p. 295.

SARETT, H. P., AND W. A. PERLZWEIG 1943 The effect of protein and B-vitamin levels of the diet upon the tissue content and balance of riboflavin and nicotinic acid in rats. J. Nutrition, vol. 25, p. 173.

SCHAEFER, A. E., J. M. MCKIBBIN AND C. A. ELVEHJEM 1942 Nicotinic acid deficiency studies in dogs. J. Biol. Chem., vol. 144, p. 679.

SNELL, E. E., AND L. D. WRIGHT 1941 Microbiological method for determination of nicotinic acid. J. Biol. Chem., vol. 139, p. 675.

VOEGTLIN, C. 1919–1920 Recent work on pellagra. The Harvey Lectures, vol. 15, p. 87.

WAISMAN, H. A., AND C. A. ELVEHJEM 1940 Multiple deficiencies in the modified Goldberger diet as demonstrated with chicks. J. Nutrition, vol. 20, p. 519.

WINTROBE, M. M. 1939 Nutritive requirements of young pigs. Am. J. Physiol., vol. 126, p. 375.

WINTROBE, M. M., R. H. FOLLIS, JR., R. ALCAYAGA, M. PAULSON AND S. HUMPHREYS 1943 Pantothenic acid deficiency in swine. With particular reference to the effects on growth and on the alimentary tract. Bull. Johns Hopkins Hosp., vol. 73, p. 313.

WINTROBE, M. M., R. H. FOLLIS, JR., M. H. MILLER, H. J. STEIN, R. ALCAYAGA, S. HUMPHREYS, A. SUKSTA AND G. E. CARTWRIGHT 1943 Pyridoxine deficiency in swine. With particular reference to anemia, epileptiform convulsions and fatty liver. Bull. Johns Hopkins Hosp., vol. 72, p. 1.

WINTROBE, M. M., M. H. MILLER, R. H. FOLLIS, JR., H. J. STEIN, C. MUSHATT AND S. HUMPHREYS 1942 Sensory neuron degeneration in pigs. IV. Protection afforded by calcium pantothenate and pyridoxine. J. Nutrition, vol. 24, p. 345.

WINTROBE, M. M., J. L. MILLER, JR. AND H. LISCO 1940 The relation of diet to the occurrence of ataxia and degeneration in the nervous system of pigs. Bull. Johns Hopkins Hosp., vol. 67, p. 377.

WINTROBE, M. M., D. M. MITCHELL AND L. C. KOLB 1938 Sensory neuron degeneration in vitamin deficiency. Degeneration of the posterior columns of the spinal cord, peripheral nerves, and dorsal root ganglion cells in young pigs fed a diet containing thiamine (B_1) and riboflavin but otherwise deficient in vitamin B complex. J. Exp. Med., vol. 68, p. 207.

WOOLEY, J. S., AND W. H. SEBRELL 1945 Niacin (nicotinic acid), an essential growth factor for rabbits fed a purified diet. J. Nutrition, vol. 29, p. 191.

THE THERAPEUTIC EFFECT OF YEAST AND PYRIDOXINE ON POIKILOCYTOSIS IN DAIRY CATTLE [1]

J. T. REID,[2] C. F. HUFFMAN AND C. W. DUNCAN
Institute of Nutrition and the Departments of Dairy and Agricultural Chemistry, Michigan State College, East Lansing

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The work of several investigators has suggested a relationship of nutrition to the occurrence of poikilocytosis in animals. The study reported in this paper was initiated for the purpose of investigating the relationship of anti-anemia mineral mixtures, yeast, pyridoxine, riboflavin and nicotinic acid to the alleviation of a poikilocytic condition found to be prevalent in some farm herds and produced experimentally in young calves by feeding semi-restricted rations. The results are of additional interest because of the absence in the literature of specific data on the production of poikilocytosis in dairy animals.

Poikilocytosis has been reported in relation to scurvy and anemia studies in guinea pigs (Hanke and Koessler, '28), anemia in suckling pigs (Kernkamp, '32), pigeons fed a vitamin B-complex deficient diet (Hogan et al., '40), anemic calves (Knoop et al., '35) and cobalt-deficient calves (Neal and Ahmann, '37).

In a recent study Reid, Huffman and Duncan ('43, '45) found a high incidence of poikilocytosis in Michigan dairy cattle accompanied by anorexia, retarded growth and general unthriftiness. Some herds manifested poikilocytosis while neighboring herds were completely unaffected. Many of the affected animals were anemic while others were normal in this respect. Since the kind and quality of rations fed to these animals varied greatly, it was believed that the condition was associated with a nutritional deficiency.

EXPERIMENTAL

Most of the data reported in this paper were derived from animals receiving a whole milk and cereal breakfast food [3] ration although many

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[2] From a thesis presented to the Graduate School of Michigan State College in partial fulfillment for the Ph.D. degree.

[3] Rice Krispies.

other calves received the individual farm ration. Some calves were treated with only one supplement, others were treated with several supplements simultaneously, while others received several supplements for varying periods but only one supplement at a time.

Two mineral mixtures, no. 1 and no. 2, were used. The no. 1 mixture supplemented the ration of seven affected calves with 250 mg. of iron, 250 mg. of manganese and 25 mg. of copper daily, while the no. 2 mixture furnished 5 mg. of cobalt daily to twenty affected calves in addition to the minerals supplied in the no. 1 mixture.

Two calves received 200 gm. of dry brewers' yeast per day while a third calf received 200 gm. and later 450 gm. of the same yeast per day. Two calves received 100 gm. of live yeast daily in addition to the no. 2 mineral mixture. Several of the B-complex vitamins were administered to ascertain the anti-poikilocytic factor(s) provided by yeast. One calf received 50 mg. of nicotinic acid per day and another calf received 25 mg. of riboflavin per day. These vitamins were administered for a period of 70 days after which time they were replaced with 40 mg. of pyridoxine daily. Pyridoxine [4] was fed to eight other calves in amounts ranging from 5 to 40 mg. per day. Some of the calves also received the no. 1 or no. 2 mineral supplement at the same time.

The microscopic examination of the red blood cells of four rumen fistula cows, all having open or unplugged fistulas, revealed the presence of from 15 to 99% poikilocytes. The condition of the most severely affected rumen fistula cow was corrected on two occasions by feeding brewers' yeast. The poikilocytic condition reoccurred, however, sometime after the yeast treatment was terminated.

The progress of recovery of the affected animals was followed by the microscopic examination of blood smears and occasionally by hanging drop preparations. Weight gains were used also as a criterion of the animal's general status of health. The hemoglobin, red blood cell count, red cell volume, and the plasma calcium, inorganic phosphorus and magnesium concentrations were determined by methods previously reported (Reid et al., '45).

Forty-two calves and four rumen fistula cows were employed in this study.

RESULTS

The results obtained from feeding the various supplementary factors in addition to the regular ration of the calves are shown in tables 1–6.

[4] The authors wish to thank Merck and Co., Inc., for furnishing a portion of the pyridoxine used.

Many animals were encountered during the course of this investigation that showed a typical anemia blood picture uncomplicated with poikilocytosis. The data obtained from calf A in table 1 illustrate a case of this type. Although the blood of most of the poikilocytosis affected animals revealed low hemoglobin concentrations, some of the animals were normal in this respect. The data for B in table 1 show a severely affected animal whose hemoglobin level is nearly normal. The data also show the loss in weight accompanying the course of a typical case of poikilocytosis when no therapeutic agent was administered.

The values included in several tables for plasma calcium, inorganic phosphorus and magnesium indicate no relationship of these constituents to the poikilocytosis anomaly.

TABLE 1

Blood picture of a calf (A) showing oligochromemia but unaffected by poikilocytosis and a calf (B) affected with poikilocytosis but not showing oligochromemia.

CALF	AGE	BODY WT.	HB	R.B.C.V.	R.B.C.C.	POIKILOCYTES	PLASMA		
							Ca	Inorg. P	Mg
no.	*days*	*kg.*	*gm. %*	*%*	*mill./mm.³*	*%*	*mg. per 100 ml.*		
A	240	103.2	6.7	18.0	5.40	0	9.9	7.02	1.24
	250	102.6	6.5	18.0	5.07	0	10.2	5.34	1.30
	260	112.5	8.0	23.0	3.97	0	11.6	7.86	1.98
	270	115.7	8.0	23.0	5.55	0	11.0	6.51	1.72
	280	116.4	9.5	24.7	6.93	0	10.9	5.70	1.57
B	80	36.7	10.9	...	...	90	11.1	6.32	1.87
	90	33.0	10.9	...	...	90	8.8	6.32	0.99
	100	33.4	10.9	...	...	95	10.2	5.34	1.03
	110	30.2	11.3	...	...	90	11.0	5.90	1.03
	120	30.0	10.3	...	...	94–97	11.1	4.96	1.01

Effect of mineral supplementation on poikilocytosis. Table 4 shows the effect of mineral supplementation of the ration of a representative animal. These data show a gradual increase in the hemoglobin concentration without any change in the severity of poikilocytosis.

Treatment with dry brewers' yeast. Table 2 shows the effectiveness of brewers' yeast in the treatment of poikilocytosis in two calves. Since these calves exhibited symptoms similar to those observed in cobalt deficiency, this element was administered for 7 weeks without response prior to the feeding of yeast. (The symptoms of cobalt deficiency disease are always alleviated within a week after the initial treatment.) The cobalt supplement was then discontinued and 200 gm. of brewers' yeast was fed daily. The distortion of the red blood cells of the calves was markedly ameliorated although no change in the level of hemoglobin

was observed. From the percentage of poikilocytes recorded in the table it appears that the distorted red cells can be restored to normally shaped corpuscles by feeding dry brewers' yeast for 3 to 4 weeks. The data indicate also the stunted condition of the calves prior to the feeding of yeast. An increased appetite and an impetus in weight gained was observed as poikilocytosis subsided. Three calves received the yeast supplement and all responded in the same manner. These findings are similar to those reported by Hogan et al. ('40) in that poikilocytosis in pigeons was corrected after 5 weeks of yeast supplementations.

TABLE 2

Effect of adding brewers' yeast to the ration.

CALF	DATE	AGE	BODY WT.	POIKILOCYTES	HB	CALF	DATE	AGE	BODY WT.	POIKILOCYTES	HB
no.		days	kg.	%	gm. %	no.		days	kg.	%	gm. %
C-3	9/24	348		75	9.8	C-4	9/24	237	....	25	11.6
	10/15	369	142.2	80	9.1		10/15	258	175.3	20	11.7
	11/13[1]	397	146.3	75–80	9.1		11/13[1]	286	179.8	23–25	11.9
	11/28	412	142.2	50–75	8.8		11/28	301	197.5	10–15	11.2
	12/18	432	165.3	5	9.6		12/18	321	202.1	0	11.7

[1] Calf received 200 gm. of brewers' yeast daily after this date.

TABLE 3

Data obtained from a representative poikilocytosis affected animal treated with live yeast.

AGE	BODY WT.	DAILY INTAKE PER KG. BODY WT.				HB	POIKILOCYTES	PLASMA		
		Fe	Cu	Mn	Co			Ca	Inorg. P	Mg
days	kg.	mg.	μg.	mg.	μg.	gm. %	%	mg. per 100 ml.		
10	36.7	0.25	43.7	13.2	3.8	6.3	75	11.5	6.19	1.84
15	Added 100 gm. live yeast and no. 2 mineral mixture daily									
15	38.9	8.86	711.6	16.2	137.7	5.7	70–75	11.2	7.10	1.69
20	41.1	8.39	673.8	18.0	147.6	7.3	20–25	11.0	7.02	2.13
30	46.2	7.47	601.0	16.6	123.0	9.2	4	11.7	4.19	1.93
40	50.5	6.86	554.5	13.7	113.0	9.8	3	11.3	9.06	1.88
50	53.6	7.79	539.7	15.6	111.6	10.7	1	11.2	7.96	1.99
60	61.4	6.91	473.6	15.5	98.1	12.6	0	11.1	8.12	1.72

Treatment with live yeast. The effect of live yeast in correcting the deformed red cell condition in calves is shown in table 3. These data show the correction of the poikilocytic condition to nearly normal-shaped erythrocytes after a 4-week period during which time the calf consumed 100 gm. of live yeast daily. A remarkable decrease in the number of poikilocytes was noted after the first and second week of yeast feeding as well as a striking transformation in the shape of the poikilocytes —

the degree of distortion became less extreme. An increased growth rate accompanied the disappearance of the poikilocytes in the blood. Only two calves were treated with live yeast but the results were uniformly the same. Since both of the calves had received the no. 2 mineral mixture simultaneously with the live yeast, it was necessary to eliminate one or the other of the supplements in order to derive the correction factor. The correction of the poikilocytic condition is attributed entirely to the ingested yeast since the mineral supplements failed to alter the extreme poikilocytosis of animals for which the data in table 4 are representative.

TABLE 4

Data obtained from a representative poikilocytosis affected animal treated with pyridoxine.

AGE [1]	BODY WT.	DAILY INTAKE PER KG. BODY WT.				HB	POIKILOCYTES	PLASMA		
		Fe	Cu	Mn	Co			Ca	Inorg. P	Mg
days	*kg.*	*mg.*	*μg.*	*mg.*	*μg.*	*gm. %*	*%*	*mg. per 100 ml.*		
50	67.3	2.52	60.4	12.0	11.9	9.0	85	11.8	6.44	2.40
70	69.8	1.29	42.6	9.9	6.9	7.0	85–90	11.9	8.56	1.18
72	Added no. 1 mineral mixture									
83	75.9	5.97	392.6	9.3	12.5	9.3	90	11.2	6.69	0.99
83	Discontinued no. 1 mineral mixture and added no. 2 mineral mixture									
100	80.1	6.26	380.6	8.9	63.9	9.5	90	11.7	5.76	1.10
120	86.4	5.09	346.3	9.3	68.5	8.9	90	11.7	6.01	1.01
137	99.1	5.73	313.7	8.1	50.9	11.6	90	11.9	7.35	2.09
137	Added 40 mg. pyridoxine daily									
140	101.6	5.73	313.7	8.1	50.9	11.6	85	11.9	7.35	2.09
150	118.9	4.21	308.6	8.0	50.0	13.3	40	11.8	6.95	1.28
160	126.8	4.03	307.3	8.5	49.8	11.3	1–5	12.2	8.56	1.14
168	134.4	3.85	300.1	7.9	47.1	10.7	0	12.2	5.74	1.03

[1] Data were obtained at 7-day intervals and on days when supplements were changed.

Treatment with pyridoxine. Eight animals varying in the severity of poikilocytosis were fed different amounts of pyridoxine. Although the daily ingestion of 5, 10, 15 or 20 mg. of pyridoxine was effective in ameliorating the poikilocytic condition, the recovery was slow. Forty milligrams of pyridoxine daily, however, resulted in the complete absence of poikilocytes within 3 or 4 weeks after treatment was begun. Tables 4–6 show the rapidity with which the poikilocytes disappeared from the blood. The disappearance of the poikilocytes was accompanied by an improvement in the general health of the animals and was also reflected by an accelerated growth rate.

Table 5 shows the ineffectiveness of feeding 25 mg. of riboflavin per day on the poikilocytosis of a representative animal. After a 70-day period, riboflavin feeding was discontinued, and 40 mg. of pyridoxine was added to the animal's ration. Although riboflavin treatment did

TABLE 5

Effects of riboflavin and pyridoxine on poikilocytosis.

DATE[1]	BODY WT.	HB	R.B.C.C.	R.B.C.V.	POIKILOCYTES	PLASMA		
						Ca	Inorg. P	Mg
	kg.	*gm. %*	*mill./mm.³*	*%*	*%*		*mg. per 100 ml.*	
4/11	48.2	8.7	9.70	25.0	5	11.3	6.87	1.65
5/9	58.2	8.7	10.72	28.0	30	12.1	8.39	1.64
5/15			Added 100 mg. Fe daily					
6/6	75.0	12.5	13.96	44.0	40	9.8	9.06	2.20
6/14			Discontinued Fe					
6/14	75.0	11.9	12.15	32.0	40	11.7	9.47	2.11
7/11	84.5	10.1	10.27	27.0	45–50	11.4	6.79	2.08
8/8	96.8	8.7	8.21	25.5	55	12.5	7.44	2.06
8/29	105.5	9.1	7.27	28.0	50	12.3	8.56	2.06
9/5			Added 25 mg. of riboflavin daily					
9/5	108.2	7.3	7.05	21.0	40	11.1	5.63	1.63
10/6	138.1	9.1	11.60	26.5	40	10.5	9.06	1.54
10/24	143.1	9.5	....	30.0	45	10.9	8.12	1.18
11/7	152.7	9.5	9.55	27.0	45	11.5	6.72	1.16
11/13	168.6		Discontinued riboflavin and added 40 mg. pyridoxine daily					
11/15	170.5	9.2	13.04	26.5	40	11.3	7.67	1.73
11/22	176.8	8.3	9.65	25.0	15	11.5	9.33	2.08
11/27	181.8	8.6	6.70	23.5	5–7	10.8	7.91	1.68
12/5	186.8	8.8	7.65	25.0	1	10.8	7.44	1.96
12/11	189.6	9.4	8.31	26.0	0	11.5	6.76	1.87

[1] Data were obtained at 7-day intervals and on days when supplements were changed.

TABLE 6

Effects of nicotinic acid and pyridoxine on poikilocytosis.

DATE[1]	BODY WT.	HB	R.B.C.C.	R.B.C.V.	POIKILOCYTES	PLASMA		
						Ca	Inorg. P	Mg
	kg.	*gm. %*	*mill./mm.³*	*%*	*%*		*mg. per 100 ml.*	
8/8	153.9	9.0	9.06	26.0	35	12.5	6.44	2.33
8/29	164.5	10.4	8.38	31.0	35	12.1	9.19	1.76
9/5			Added 50 mg. nicotinic acid daily					
9/5	171.5	9.8	...	26.5	30	11.3	7.86	2.10
10/6	187.5	9.8	7.24	29.5	30–35	11.1	7.67	1.73
10/24	195.0	9.0	...	28.0	35	10.9	7.14	2.11
11/7	205.1	9.4	6.95	26.0	45	11.2	6.57	1.37
11/13	213.7		Discontinued nicotinic acid and added 40 mg. pyridoxine daily					
11/15	218.5	9.0	6.50	26.0	40	11.4	6.79	1.35
11/22	221.6	8.7	8.05	24.0	15	11.2	6.87	2.05
11/27	226.6	8.7	5.81	24.5	7	11.2	5.19	2.15
12/5	231.48	9.1	7.86	25.5	1	9.9	4.77	2.02
12/11	241.36	9.1	7.15	25.0	0	10.9	6.51	2.19

[1] Data were obtained at 7-day intervals and on days when supplements were changed.

not affect the number of poikilocytes, the pyridoxine treatment corrected the condition to normal.

Table 6 shows that 50 mg. of nicotinic acid daily for 70 days failed to correct poikilocytosis in a representative animal, whereas the transformation to normal was attained after treatment with 40 mg. of pyridoxine daily. It should be emphasized that the decreased rate of growth and the poikilocytic status of the animals remained unchanged during the mineral supplement, riboflavin and nicotinic acid feeding periods, but rapid response was obtained when pyridoxine was added to the ration.

DISCUSSION

Different agents known to stimulate hemapoiesis were fed in an effort to correct the poikilocytic status of calves and rumen fistula cows. The mineral supplements had no effect on poikilocytosis although a noticeable elevation in the hemoglobin level resulted from their supplementation. The feeding of either brewers' yeast or live yeast was effective in ameliorating the poikilocytic condition within 3 to 4 weeks.

Several of the B-complex vitamins contained in yeast were used to treat the poikilocytic animals in an attempt to derive the curative factor(s) for this anomaly. The use of riboflavin and nicotinic acid did not alter the shape of the poikilocytes but pyridoxine administration resulted in a specific response. In cases where severe poikilocytosis and retarded growth were found, the ingestion of pyridoxine resulted in an increase in appetite and an accelerated growth rate coincident with the subsidence of the poikilocytes. In the less severe cases the increase in the rate of growth after the administration of pyridoxine was not appreciable. Although the daily ingestion of 5 to 20 mg. of pyridoxine brought about slow recovery, the ingestion of 40 mg. accelerated the complete restoration of the poikilocytes to normal erythrocytes. Recovery was effected within 3 to 4 weeks. The prognosis of the animals treated with 40 mg. of pyridoxine was excellent and the cure was permanent.

In most of the cases observed, young calves were the animals most commonly affected with poikilocytosis whereas the incidence in mature animals was quite limited. Observation was made also of the apparently spontaneous recovery of previously affected calves. It seems logical to suppose that recovery occurred concomitant with the development of the rumen flora and fauna.

Since the blood of four rumen fistula cows revealed the presence of poikilocytes, the existence of the open rumen fistulas offered the possi-

bility that a derangement in ruminal function may have been responsible for the occurrence of the poikilocytes. Since the young calves receiving a restricted ration of whole milk and a cereal breakfast food generally manifested poikilocytosis and the older animals did not, it appeared that a deficiency of some factor(s) necessary for the maintenance of corpuscular integrity may be synthesized in the normal mature rumen. The early studies of Theiler et al. ('15) and Bechdel et al. ('26, '27) demonstrated that cattle were healthy and normal on vitamin B-complex deficient rations, while rumen synthesis of the complex was reported by Scheunert and Schieblich ('23), Bechdel et al. ('28) and Wegner and coworkers ('40, '41). The latter group of investigators believed that the increase in B-vitamins in the rumen ingesta, as compared to the ration fed, was due to synthesis and not to a concentration effect. Arnold and Elvehjem ('40) stated that ruminant animals are able to satisfy their requirements for at least some members of the B-complex by virtue of the fermentation which occurs in the digestive tract. The synthesis of pyridoxine by the bacteria in the rumen has been pointed out by several workers (McElroy and Goss, '40). Wegner and coworkers ('40, '41) showed that the pyridoxine content of the rumen ingesta is higher than that of the ration fed and concluded that the increase was due to bacterial synthesis. These findings support the earlier work of McElroy and Goss ('40) in that the vitamin B_6 potency of the rumen contents is 6 to 8 times as great as that found in the ration. These investigators favored a true synthesis of the vitamin related to growth of the rumen flora rather than a concentration caused by differential rates of passage of different nutrients from the rumen. No mention was made as to whether the rumen fistulas were open or closed. They showed that a normal amount of vitamin B_6 is found in the milk of a cow on a vitamin B_6 deficient ration which seems to indicate that the cow was able to synthesize enough of this vitamin to satisfy her maintenance needs and in addition transfer a normal amount into the milk.

No reports concerning the rumen synthesis of pyridoxine have indicated that the rumen of young calves has flora developed sufficiently to synthesize this vitamin in quantity large enough to satisfy the animal's maintenance requirements or that the vitamin is synthesized at all. It seems logical, therefore, to suppose that the ration fed, the age of the calf, and the condition of the rumen of both rumen fistula cows and normal calves are factors concerned in the synthesis of pyridoxine.

Although no known case of vitamin B_6 deficiency in cattle has been reported, a deficiency of this vitamin has been reported in other animals.

Fouts and associates ('38) successfully treated a severe microcytic hypochromic anemia in dogs with vitamin B_6 after iron therapy had failed to relieve the anemia. Later Fouts et al. ('40) showed that adult dogs as well as puppies developed vitamin B_6 deficiency anemia which did not respond to iron and copper therapy. McKibbin and coworkers ('39) reported that the ingestion of a low level of vitamin B_6 is necessary for growth but the ingestion of a higher level is necessary to protect pyridoxine deficient dogs against hypochromic microcytic anemia. They suggested 60 μg. of vitamin B_6 as an anemia-curative dose for dogs.

The ability of pyridoxine to stimulate the hemapoietic system of deficient patients has been extensively demonstrated by increased blood regeneration and the restoration of the red corpuscles to normal size. The vitamin was used by Vilter and associates ('40) to treat three pellagrins with macrocytic anemia and two patients with pernicious anemia. A subjective relief and a slight reticulocytosis occurred. Although these investigators stated that vitamin B_6 definitely affects the hemapoietic system of humans who have the macrocytic anemia of pellagra or pernicious anemia, they did not imply that it is an antipernicious anemia factor. It should be emphasized, however, that pernicious anemia is one of the diseases constantly associated with poikilocytosis. Wintrobe et al. ('43) produced a severe anemia in pigs by feeding a pyridoxine deficient diet. When pyridoxine was administered, a rapid regeneration of blood accompanied the restoration of normal corpuscular size. The reports in the literature indicate that simple stomach animals and humans may suffer from a lack of vitamin B_6 whereas a deficiency of this vitamin in the normal ruminant is unlikely to occur by virtue of bacterial synthesis. Poikilocytosis, however, has not been associated with pyridoxine deficiency in simple stomach animals.

This investigation has shown that when poikilocytes do occur in the blood of calves they can be replaced with normal erythrocytes following the administration of brewers' yeast, live yeast or vitamin B_6. The results of this study have indicated also the possibility of a lack of or a derangement of the normal rumen activity necessary for the synthesis or utilization of pyridoxine either for the direct maintenance of corpuscular integrity or for the factor(s) responsible for this function.

SUMMARY

Young calves on semi-restricted rations and cows with large open fistulas of the rumen were found to be the dairy animals most commonly affected with poikilocytosis. Dry brewers' yeast, live yeast, or pyridoxine were effective therapeutic agents for the treatment of this condi-

tion, whereas the ingestion of nicotinic acid, riboflavin or a mineral mixture containing iron, copper, cobalt and manganese did not elicit any curative effects on the disease.

The occurrence and the degree of severity of poikilocytosis were independent of the hemoglobin content, red blood cell count, red blood cell volume and the plasma concentration of calcium, inorganic phosphorus and magnesium.

As a result of this investigation the hypothesis is offered that the primary defect responsible for the occurrence of poikilocytosis in dairy animals is due to a lack of or an interference with normal ruminal activity.

LITERATURE CITED

ARNOLD, A., AND C. A. ELVEHJEM 1940 The relation of the vitamin B complex to the nutrition of domestic animals. J. Am. Med. Assn., vol. 96, p. 56.

BECHDEL, S. I., C. H. ECKLES AND L. S. PALMER 1926 Vitamin B requirement of the calf. J. Dairy Sci., vol. 9, p. 409.

BECHDEL, S. I., AND H. E. HONEYWELL 1927 The relation between the vitamin B contents of the feed eaten and of the milk produced. J. Agric. Research, vol. 35, p. 283.

BECHDEL, S. I., H. E. HONEYWELL, R. A. DUTCHER AND M. H. KNUTSEN 1928 Synthesis of vitamin B in the rumen of the cow. J. Biol. Chem., vol. 80, p. 231.

FOUTS, P. J., O. M. HELMER AND S. LEPKOVSKY 1940 Nutritional microcytic hypochromic anemia in dogs cured with crystalline factor I. Am. J. Med. Sci., vol. 199, p. 163.

FOUTS, P. J., O. M. HELMER, S. LEPKOVSKY AND T. H. JUKES 1938 Production of microcytic hypochromic anemia in puppies on synthetic diet deficient in rat antidermatitis factor (vitamin B_6). J. Nutrition, vol. 16, p. 197.

HANKE, M. T., AND K. K. KOESSLER 1928 The effect of scurvy-producing diets and tyramine on the blood of guinea pigs. J. Biol. Chem., vol. 80, p. 499.

HOGAN, A. G., L. R. RICHARDSON, P. E. JOHNSON AND R. N. NISBET 1940 Pigeon anemia as a deficiency disease. J. Nutrition, vol. 20, p. 203.

KERNKAMP, H. C. H. 1932 The blood picture of pigs kept under conditions favorable to the production and to the prevention of so-called "anemia of suckling pigs." Minn. Agr. Exp. Sta. Tech. Bull. 87.

KNOOP, C. E., W. E. KRAUSS AND R. G. WASHBURN 1935 The development of nutritional anemia in dairy calves. J. Dairy Sci., vol. 18, p. 337.

MCELROY, L. W., AND H. GOSS 1940 A quantitative study of vitamins in the rumen contents of sheep and cows fed vitamin-low diets. II. Vitamin B_6 (pyridoxine). J. Nutrition, vol. 20, p. 541.

MCKIBBIN, J. M., R. J. MADDEN, S. BLACK AND C. A. ELVEHJEM 1939 The importance of vitamin B_6 and factor W in the nutrition of dogs. Am. J. Physiol., vol. 128, p. 102.

NEAL, W. M., AND C. F. AHMANN 1937 The essentiality of cobalt in bovine nutrition. J. Dairy Sci, vol. 20, p. 741.

REID, J. T., C. F. HUFFMAN AND C. W. DUNCAN 1943 A study of poikilocytosis in dairy cattle. J. Dairy Sci., vol. 26, p. 738.

———— 1945 Poikilocytosis in dairy cattle. Arch. Path., vol. 39 (6), p. 351.

SCHEUNERT, A., AND M. SCHIEBLICH 1923 Zur Kenntnis der Vitamine. Über die Bildung von Vitamin B durch obligate Darmbakterien. Biochem. Z., Bd. 139, S. 57.

THEILER, A. H., H. H. GREEN AND P. R. VILJOEN 1915 Contribution to the study of deficiency disease with special references to the lambzietkeproblem in South Africa. Union South Africa Dept. Agr. Research Repts. 3–4, Dir. Vet. Res., p. 3.

VILTER, R. W., H. S. SCHIRO AND T. D. SPIES 1940 Effect of synthetic vitamin B_1 on the hemapoietic system of human beings. Nature, vol. 145, p. 388.

WEGNER, M. I., A. N. BOOTH, C. A. ELVEHJEM AND E. B. HART 1940 Rumen synthesis of the vitamin B complex. Proc. Soc. Exp. Biol. Med., vol. 45, p. 769.

———— 1941 Rumen synthesis of the vitamin B complex on natural rations. Proc. Soc. Exp. Biol. Med., vol. 47, p. 90.

WINTROBE, M. M., R. H. FOLLIS, JR., M. H. MILLER, H. J. STEIN, R. ALCAYAGA, S. HUMPHREYS, A. SUKSTA AND C. E. CARTWRIGHT 1943 Pyridoxine deficiency in swine with particular reference to anemia, epileptiform convulsions and fatty liver. Bull. Johns Hopkins Hosp., vol. 72, p. 1.

FIELD ILLUMINATION AND COMMERCIAL HANDLING AS FACTORS IN DETERMINING THE ASCORBIC ACID CONTENT OF TOMATOES RECEIVED AT THE CANNERY

G. FRED SOMERS, KARL C. HAMNER AND WALTER L. NELSON

Agricultural Research Administration, U. S. Department of Agriculture, U. S. Plant, Soil and Nutrition Laboratory, Ithaca, New York; School of Nutrition,[1] Cornell University, Ithaca

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A recent resolution by the American Medical Association's Council on Food establishes for tomato juice a minimum level of 20 mg. of ascorbic acid per 100 ml. to meet the "Accepted" seal of the Association. The establishment of such a standard raises the question as to whether all producers of tomato juice could possibly attain such a level with any degree of consistency. Many studies have indicated the wide variations in the ascorbic acid content of tomatoes and tomato products, but a correlated study of the several factors operating in a specific location has been lacking in most of the reports. It is possible that in certain regions an ascorbic acid level of this magnitude could not be obtained at the present time, even when favorable commercial varieties and good canning management were given primary consideration. For a number of years, the laboratories participating in this investigation have been interested in various aspects of this problem, and the present paper is a report of a correlated study.

Numerous investigators have reported on various factors which influence the ascorbic acid content of tomatoes and tomato products. In a review of the literature, Hamner and Maynard ('42) concluded that fruit development and nutrition of the plant had little effect on the ascorbic acid content of the fruit. Varietal differences of from 13 to 44 mg. per 100 gm. of fruit were reported, and light intensity, season and location, and processing were indicated as factors which markedly influence the ascorbic acid value of the fruit or the product made from the fruit.

Some recent studies by Wade ('42), Renard and Kanapaux ('42), and Kidson ('43) have confirmed the findings that wide differences in as-

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corbic acid content of tomato fruit exist among various varieties. Hamner, Lyon, and Hamner ('42) found that winter-grown fruits had approximately 50% as much ascorbic acid as fruits from plants of the same variety grown in the summer. Holmes, Jones and Ritchie ('43) reported slightly greater differences between winter tomatoes and fully ripe summer tomatoes, than those reported by Hamner et al. The studies of Murphy ('42) and Kidson ('43) have also indicated the large effect that environmental conditions exert on the ascorbic acid content of fruits grown in various localities. Hamner, Bernstein and Maynard

TABLE 1

Ascorbic acid content of commercially canned tomato juice.

SOURCE	NUMBER OF CANS	ASCORBIC ACID		SOURCE	NUMBER OF CANS	ASCORBIC ACID	
		Mean ± SD [1]	Range			Mean ± SD	Range
Market		*(mg./100 ml.)*	*(mg./100 ml.)*	Cannery [2]		*(mg./100 ml.)*	*(mg./100 ml.)*
Brand 1	5	11.9 ± .54	11.3–12.5	Lot 1	10	16.3 ± .82	15.3–17.8
Brand 2	10	12.1 ± 1.07	10.6–13.9	Lot 2	10	17.7 ± 1.64	15.2–19.3
Brand 3	10	5.7 ± .27	5.0– 6.0	Lot 3	10	16.5 ± .88	15.1–17.5
Brand 4	10	5.7 ± .26	5.4– 6.2	Lot 4	10	17.4 ± .54	16.7–18.4
Brand 5	10	17.8 ± .66	17.0–18.7	Lot 5	10	21.0 ± .09	20.6–21.6
Brand 6	10	6.9 ± .57	6.4– 8.0	Lot 6	10	20.6 ± .88	19.4–21.5
Brand 7	10	7.9 ± 3.92	4.6–13.4	Lot 7	10	19.8 ± 1.52	17.5–21.2
Brand 8	10	14.5 ± .76	13.8–16.1	Lot 8	10	18.5 ± .60	17.7–19.7
				Lot 9	10	19.8 ± .44	19.1–20.4
				Lot 10	10	18.2 ± .28	17.8–18.6
				Lot 11	10	19.3 ± .85	17.8–20.1
				Lot 12	10	18.7 ± .25	18.4–19.0
				Lot 13	10	18.2 ± .85	16.9–19.1
				Lot 14	10	16.2 ± .44	15.6–16.9
				Lot 15	10	19.4 ± 1.26	17.8–22.4
				Lot 16	10	19.1 ± 1.20	16.8–20.4

[1] Standard Deviation = $\sqrt{Sd^2/n-1}$.

[2] The manufacturing process is that of Factory A described by Robinson et al. ('45).

('45) have shown the great influence on the ascorbic acid content of tomatoes produced by variations in light intensity previous to harvest. Such factors as degree of ripeness after the fruit is "mature green," storage of fruit for 10 to 14 days at temperatures from 65° to 90°F., fertilizer treatment, and relative humidity had only a slight effect, if any, on the ascorbic acid content of the fruit.

The work of Bailey ('38) indicated that market tomato juice varies widely in ascorbic acid content, but did not show the extremely low values that have been encountered in other surveys. Recently, Pressley, Ridder, Smith and Caldwell ('44) reported a range of 2.5 mg. to 25.2 mg. per 100 gm. for commercially canned tomato juice. However,

in these studies no data were given relative to the prior treatment of the juice, the exact source of the raw material, or the variation from can to can within a particular brand. Therefore, the factors mainly responsible for the large variations could not be ascertained.

Preliminary investigations carried out in this laboratory on the ascorbic acid content of market tomato juice (table 1) showed that a relatively small can to can variation occurred within a single brand but that wide variations existed among various brands. A survey of the ascorbic acid content of the tomato juice produced at a single cannery [2] also was made. Analyses were made of the juice produced each day the plant was in operation throughout the season. The results, which are presented in table 1, show little can to can variation for any one day, but a somewhat greater variation among days. Even so, the total variation throughout the season was not great as compared to the variation between brands on the retail market. These results indicate that, even with a uniform canning process, there is a considerable variation in the ascorbic acid content of tomato juice. It seems unlikely that this would arise during the processing since the same process was used throughout (see also the accompanying paper by Robinson et al., '45). On the other hand it seems likely that the ascorbic acid content of the tomatoes supplied to the cannery may be a factor. If this is so, variation in the tomatoes may arise from two sources: (1) losses subsequent to picking and before processing, (2) differences in the ascorbic acid content of tomatoes in the field. Some preliminary studies have been made of this aspect of the problem and the following is a report on the findings. At the same time a study was made of the influence of various processing procedures. In this latter study, which was made by Robinson et al. ('45, see accompanying paper), the same source of tomatoes was used, in part, as in the first two experiments in this paper.

Loss of ascorbic acid from the field to the cannery

Experiment 1. On August 31st, the above-mentioned cannery was scheduled to utilize Stokesdale tomatoes from a particular field a short distance away from the cannery. Pickers entered the field in the morning and proceeded to harvest the crop in the usual manner. The following samples were collected between 8:30 and 9:15 A.M. Sample no. 1: Fully ripe, uninjured fruits picked from the vines a short distance

[2] The raw material used by this cannery is discussed in this paper. The type of process used is that of Factory A as described by Robinson et al. ('45) in an accompanying paper. Grateful acknowledgment is made to the assistance and cooperation of Mr. Wilson of the Empire State Pickling Co., Phelps, New York.

ahead of the commercial harvesters. Sample no. 2: Fruit from the boxes into which the commercial pickers were emptying their baskets. In this sample, fruits were selected which showed no injury or which were only very slightly damaged. Sample no. 3: A random sample from these boxes. Damaged as well as uninjured fruit were taken in an effort to get a sample as nearly representative of the harvest as possible. Sample no. 4: Since the portion of the field which was being harvested was infested with weeds, a sample of fruit was taken from an adjacent portion of the same field which had been carefully weeded. All the fruits for this sample were taken from the south side of the vines where the fruits and the adjacent leaves had maximum exposure to sunlight.

The cannery commenced processing fruit from this field about 2 P.M. An additional sample was, therefore, taken at the canning plant as follows. Sample no. 5: A representative sample taken at random from the boxes on the floor of the cannery.

For each sample twenty to thirty fruits were taken, individually wrapped in a large amount of tissue paper and carried to Ithaca for analysis the next day. They were stored overnight at about 40°F. Some of the fruits were soft at the time of analysis. They were all analyzed nevertheless. The fruits of samples 1 and 2 arrived in excellent condidition. Evidence from previous work by Hamner et al. ('45), has indicated that the method of harvesting and preparing samples 1 and 2 should not have resulted in any appreciable loss in ascorbic acid.

The method of obtaining the samples of individual fruits and of preparing the acid extract has been previously described (Hamner et al., '42). The samples of the fruit were extracted with a mixture of 2% metaphosphoric acid in N sulfuric acid and ascorbic acid determinations made by a modified xylene method (Bukatsch, '39; Stotz, '41; Nelson and Somers, '45). The results of the ascorbic acid analyses are shown in table 2. There was no significant difference in ascorbic acid content between any of the samples from 1 to 5. Robinson and his co-workers ('45), took samples from the trimming line only a few minutes after our sample no. 5 was taken from the boxes in the canning plant. Their samples were extracted within a few minutes after the sample was taken and were analyzed a few hours later. The ascorbic acid value obtained did not differ significantly from the values obtained in our samples 1 to 5, i.e., they obtained 31 mg. of ascorbic acid per 100 gm. of fresh fruit on this sample. It would appear, therefore, that there was no appreciable loss in ascorbic acid from the field to the trimming line in the cannery in this experiment.

Experiment 2. On September 18th, the cannery was processing the same variety of tomato from another field about half a mile from the above field. A sample of thirty-one fruits collected in the morning from this field gave an ascorbic acid value of 28.5 ± 0.75 mg. of ascorbic acid per 100 gm. A sample of twenty fruits taken at random from the boxes in the canning plant late in the afternoon and extracted immediately for ascorbic acid gave a value of 24.1 ± 0.90 mg., and another sample of twenty fruits taken from the trimming line and extracted in acid at once gave a value of 24.0 ± 1.08 mg.

TABLE 2

Ascorbic acid content (milligrams per 100 gm. fresh fruit) of tomatoes prior to processing.

SAMPLE NO.	DESCRIPTION OF SAMPLE	NO. OF FRUITS	ASCORBIC ACID Mean ± Standard Error
1	Selected ripe fruit from vine	23	30.8 ± 1.26
2	Selected fruit from boxes in field	23	33.2 ± 1.08
3	Random sample from boxes in field	20	32.9 ± 1.17
4	Fruit selected which were exposed to maximum illumination in the weeded portion of the field	21	31.8 ± 0.90
5	Random sample from boxes in canning plant	25	29.0 ± 0.94

In this latter experiment, it appears that there was a significant loss in ascorbic acid between the field and the processing line. It may be recalled that sample no. 5 (table 1) taken from the boxes on the floor of the canning plant was lower in ascorbic acid than the field samples, although not significantly so. It seems possible, therefore, that one might expect some loss in ascorbic acid content of tomatoes during the picking, transportation, and holding of the fruit at the cannery before processing. If the fruits are held overnight or more than a day at the cannery before processing, losses might be considerable, especially if the fruits are injured and in poor condition.

Variations in ascorbic acid content of fresh tomatoes during the canning season and from one field location to another

Since a study was made of variations in ascorbic acid content of canned tomato juice during the course of the canning season at this particular cannery, measurements of the ascorbic acid content of fresh tomatoes from the field were also made during the course of the season.

Thirty ripe fruits, representing a random sampling of the field, were picked on three different dates, one relatively early in the canning season, another at the height of the season, and a third near the end. The ascorbic acid values in milligrams per 100 gm. fresh fruit with the corresponding dates were as follows: August 31st, 30.8 ± 1.26; September 7th, 29.4 ± 0.82; and September 18th, 31.4 ± 1.25. These data are too few to warrant definite conclusions, but they do indicate that there was no general trend in ascorbic acid values during the course of the season, either upward or downward.

The cannery, during the course of the season, obtained fruit from many different fields within a radius of a few miles. Hence it was of interest to see how much variation in ascorbic acid might be found between samples of fruit of the same variety collected from different fields. It was also desirable to determine whether or not such variations as might be found could be correlated with the amount of illumination at each field, since some previous results (Hamner et al., '45) have indicated that the light intensity to which tomato plants are exposed may to a large extent determine the ascorbic acid level in the fruit.

Three fields of tomatoes were selected: one containing Stokesdale tomatoes at Phelps, New York (the field which had been used in connection with the cannery experiments); one with both Stokesdale and John Baer tomatoes at Geneva, New York; and one with John Baer tomatoes at Lincoln, New York.[3]

An integrating light recorder similar to that described by Sprague and Williams ('41) was placed within 0.5 of a mile of each of these fields. These recorders had been calibrated previously at the laboratory, and at the end of the experiment they were again calibrated. It is believed that the comparative values are reasonably accurate. The instrument consists of a photoelectric cell mounted in a spherical globe and an instrument which records a number of counts proportional to the amount of illumination (Sprague and Williams).

The light recorders were in operation for a period of 18 days from September 1st to 18th, inclusive, and daily records were obtained. The following are the comparative values for the entire period: Phelps $= 100$, Geneva $= 67.4$, and Lincoln $= 71.8$. On September 18th, analyses for ascorbic acid content were made on fruits from each of the three fields with the results shown in table 3. The amount of illumination at Geneva was 67.4% of that at Phelps, and the ascorbic acid values for the Stokesdale variety were 75% of those at Phelps. There was almost

[3] Grateful acknowledgment is made to the cooperation of Dr. C. B. Sayre in obtaining these samples.

the same illumination at Lincoln as at Geneva, and the ascorbic acid values were almost identical for the John Baer variety.

The illumination values given above represent average values over the entire experimental period. The daily records showed similar fluctuations at Phelps and Geneva, although the values at Phelps were nearly always slightly higher than those at Geneva on any particular day. The fluctuations from day to day at Lincoln, which is nearly 30 miles northwest of Phelps, followed a somewhat different pattern than at the other two locations. It was surprising that there was so much difference in illumination between Geneva and Phelps which were only 8 miles apart. Geneva is located at the northern end of Seneca Lake, while Phelps is northwest of Geneva. It may be that the proximity of Seneca Lake resulted in the lower illumination values at Geneva.

TABLE 3

Ascorbic acid content and relative illumination of tomatoes grown at three fields.

LOCATION	VARIETY	RELATIVE ILLUMINATION	NO. OF ANALYSES	ASCORBIC ACID CONTENT OF FRESH FRUIT
				Mean ± Standard Error
				mg./100 gm.
Phelps, N. Y.	Stokesdale	100	27	31.4 ± 1.25
Geneva, N. Y.	Stokesdale	67.4	42	23.6 ± 0.65
	John Baer		42	20.0 ± 0.40
Lincoln, N. Y.	John Baer	71.8	27	19.0 ± 0.59

These data indicate that the variations in ascorbic acid content from one field to another may reflect variations in illumination. The results are in accordance with previous results (Hamner et al., '42; Murphy, '42; Hamner and Maynard, '42) which indicate that the variations in ascorbic acid values from one location to another may be very large and that the intensity of illumination may be the dominant factor in causing the variations (Hamner et al., '45).

DISCUSSION

In considering the losses in nutritive value which might occur during processing of food products, one should bear in mind the possible losses during the picking and handling of the crop prior to its arrival and utilization at the cannery. In comparing the nutritive value of products from various canneries, it is also important to ascertain to what extent the values obtained for a given product are determined by the original values of the fresh material which was used. For example, it seems

possible that the relatively great variations which have been reported for the ascorbic acid values of fresh tomatoes might account for much of the variation which occurs in canned tomato juice.

In this paper, wide variations in ascorbic acid content of canned tomato juice obtained on the market were found. These variations are much greater than can be accounted for on the basis of the experimental variations in the processing methods (see the accompanying paper by Robinson et al., '45). The loss in ascorbic acid during the picking and transportation of the fruit to the cannery was so small in these experiments that it would seem likely that variations in these procedures would not explain the great differences which are found in juice from several sources. The limited storage studies do not provide evidence that variations in storage time or conditions could explain the variable market values.

The work presented here, as well as previous investigations (Hamner et al., '42, '45; Murphy, '42; Hamner and Maynard, '42), indicates that the location or season at which the fruits are produced causes very great variations in the ascorbic acid content of fresh tomatoes. These variations are of sufficient magnitude to provide a possible explanation of the variations in market tomato juice. Differences between varieties doubtless provide for part of the variations.

SUMMARY

1. Little loss in ascorbic acid in tomatoes was found during the picking and transporation of the fruit to the cannery.

2. Considerable variation in ascorbic acid content was found from one tomato field to another.

3. The data presented indicate that the amount of light for 18 days prior to harvest is closely correlated with the ascorbic acid content of tomatoes under field conditions.

4. Differences in amount of illumination appear to account for much of the variation in ascorbic acid content of tomatoes which is found between fields several miles apart.

5. It is suggested that the variations in the fresh tomatoes may be an important factor in determining the variations observed in the ascorbic acid content of market tomato juice.

LITERATURE CITED

BAILEY, E. M. 1938 Tomato juice, canned. Conn. Agr. Exp. Sta. Bull. no. 415, p. 698.

BUKATSCH, F. 1939 Eine neue Methode des C-Vitamins mit dem Stufenphotometer von Zeiss. Z. Physiol. Chem., Bd. 262, S. 20.

HAMNER, K. C., LEON BERNSTEIN AND L. A. MAYNARD 1945 Effects of light intensity, day length, temperature and other environmental factors on the ascorbic acid content of tomatoes. J. Nutrition, vol. 29, p. 85.

HAMNER, K. C., C. B. LYON AND C. L. HAMNER 1942 Effect of mineral nutrition on the ascorbic acid content of the tomato. Bot. Gaz., vol. 103, p. 586.

HAMNER, K. C., AND L. A. MAYNARD 1942 Factors influencing the nutritive value of the tomato. U.S.D.A. Misc. Pub., no. 502.

HOLMES, A. D., C. P. JONES AND WALTER S. RITCHIE 1943 The ascorbic acid content of late winter tomatoes. New Eng. J. Med., vol. 229, p. 461.

KIDSON, ELSA B. 1943 Vitamin C content of different tomato varieties grown in the Nelson District. New Zealand J. Sci. Tech., 25 B, p. 129.

MURPHY, ELIZABETH F. 1942 The ascorbic acid content of different varieties of Maine grown tomatoes and cabbages as influenced by locality, season, and stage of maturity. J. Agr. Res., vol. 64, p. 483.

NELSON, WALTER L., AND G. FRED SOMERS 1945 The application of the xylene method to the determination of ascorbic acid in large numbers of tomato and tomato juice samples. Ind. Eng. Chem., Anal. Ed. In press.

PRESSLEY, ANNE, CLARA RIDDER, M. C. SMITH AND EMILY CALDWELL 1944 The nutritive value of canned foods. II. Ascorbic acid and carotene or vitamin A content. J. Nutrition, vol. 28, p. 107.

REYNARD, G. B., AND MARGARET S. KANAPAUX 1942 Ascorbic acid (vitamin C) content of some tomato varieties and species. Proc. Am. Soc. Hort. Sci., vol. 41, p. 298.

ROBINSON, W. B., ELMER STOTZ AND Z. I. KERTESZ 1945 The effect of manufacturing methods on the ascorbic acid content and consistency characteristics of tomato juice. J. Nutrition, vol. 30, p. 435.

SPRAGUE, V. G., AND E. M. WILLIAMS 1941 An inexpensive integrating light recorder. Plant Physiol., vol. 16, p. 629.

STOTZ, ELMER 1941 A clinical method for the determination of ascorbic acid in blood plasma and urine. J. Lab. and Clin. Med., vol. 26, p. 1542.

WADE, B. L. 1942 The relationship of variety and environment to the nutrient content of vegetables grown in the South. Assn. Southern Agr. Workers, Proc. Ann. Convention, vol. 43, p. 168.

THE EFFECT OF MANUFACTURING METHODS ON THE ASCORBIC ACID CONTENT AND CONSISTENCY CHARACTERISTICS OF TOMATO JUICE[1, 2]

WILLARD B. ROBINSON, ELMER STOTZ AND Z. I. KERTESZ
New York State Agricultural Experiment Station, Cornell University, Geneva, New York

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The accompanying paper by Somers, Hamner and Nelson ('45) illustrates the great variations which may be found in the ascorbic acid content of market samples of tomato juice, and the relation of these variations to growing conditions, harvesting, and delivery of tomatoes to the processing plant.

The ascorbic acid level of tomato juice purchased on the market will be governed by three main factors, namely (1) the ascorbic acid content of the raw product, (2) the effect of production methods, and (3) the influence of storage on the finished product. This paper presents the results of studies on ascorbic acid losses during processing in two commercial canning plants, with special reference to the preheating temperatures employed during crushing of the tomatoes and to related changes in the consistency of the juice produced. In this paper the term "breaking" refers to the crushing of tomatoes prior to extraction of the juice, "hot break" when heat is simultaneously applied during the crushing, "cold break" when no added heat is employed. Storage studies of the tomato juice produced under controlled conditions are in progress and will be presented in another report.

There are several older references to the ascorbic acid losses which may occur in the preparation of tomato juice. Some of these investigators employed insufficiently accurate bioassay methods for determination of the vitamin and most of the studies were not carried out under commercial production conditions. The general conclusion is prevalent, however, that the processing loss is small when conditions avoiding oxidation are observed (Kohman, Eddy and Gurin, '33; Daggs and Eaton, '34). Sanborn ('38) has given particular emphasis to the

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increased losses which may occur when the hot juice is exposed excessively to aeration, for example during centrifugal pumping and tank holding of the juice. One of the few actual figures reported in this survey cites a 19% loss of ascorbic acid during a 13-minute period required for the heating of juice in a tank prior to can filling. Sanborn stated that the most complete conservation of vitamin C was found in plants employing the "hot break" of the tomatoes.

Kertesz and Loconti ('44) have studied the factors which govern the consistency of tomato juice, and demonstrated that application of the "hot break" method gives a juice of superior consistency. The wide use of tomato juice as an important nutritional source of vitamin C is contingent on maintaining satisfactory color, consistency, and flavor in the juice. Since considerations of juice consistency advise the "hot breaking" of tomatoes, it was important to determine whether the elevated temperature (up to 200°F.) employed would cause more loss of ascorbic acid than if the juice were prepared without heat or with only preheating. Opposed to the possibility of increased destruction due to elevated temperatures, the presence in the tomato skin of thermo-labile agents which facilitate the destruction of ascorbic acid (Wokes and Organ, '43), could be suspected of causing more rapid loss of ascorbic acid during the manufacture by a "cold break" method. Particular attention was therefore given in this study to the effect of preheating temperatures on the ascorbic acid content of the processed tomato juice. Simultaneous observations on the consistency of these juices were made to determine whether any relation exists between consistency and the stability of the ascorbic acid.

The experiments were carried out in two commercial canning plants during regular production in the 1944 season.[3] One of these employed the "hot break" procedure and here it was possible to study the effect of different preheating temperatures without other changes in the procedure. In the second plant where the "cold break" process was used, conditions favored a study of the effect of tank holding and processing times on the ascorbic acid content of the finished juice.

EXPERIMENTAL PROCEDURES

The manufacturing procedure used in factory A was as follows: The tomatoes were washed, sorted, and trimmed in the usual manner. From the sorting table the fruit entered a crusher-preheater.[4] This

[3] We are grateful to Mr. Floyd Wilson of the Empire State Pickling Co., Phelps, New York, and to Mr. Irving B. Cook of the G.L.F. plant in Waterloo, New York, for their assistance and cooperation.

[4] Food Machinery Corp., 40-gallon per minute capacity.

was of tubular construction and contained a worm revolving at 184 RPM which crushed the tomatoes and forced them through the tube. With a given rate of steam supply to the preheater, the extent of heating depended somewhat on the volume of tomatoes. Preheating temperatures were varied by regulating the steam supply to the breaker, and recorded by some 40 to 60 temperature readings on the material emerging from the breaker. The crushed tomatoes then entered a juice extractor [5] in which the juice was pressed through a closely fitting screen by a screw revolving at 300 RPM. The juice was pumped into a 200-gallon stainless steel tank, heated to 180°F., and filled into no. 2 size cans. These were sealed on a high speed closing machine, processed for 5 minutes in a tank of hot water (190°F.), and finally cooled to about 130°F.

In factory B the tomatoes were trimmed, crushed, and the juice extracted entirely without the application of heat. The juice was pumped into 600-gallon stainless steel tanks and then heated to 180°F. with continuous stirring. Usually the juice was held for at least 15 minutes before filling into no. 3 (tall) size cans. These were processed for 20 minutes in boiling water and cooled for several hours in a cold water tank.

Temporary interruptions in the production line made it possible to relate all samples taken along the processing line to the particular lot of fruit used as raw product. In practice, the preheater, juicer, and a receiving tank were emptied before starting a given temperature run. A batch of tomatoes, all collected from the same field was then prepared and fed to the preheater, and approximately thirty whole fruits removed from the trimming line at random (series A, table 1) during the 20 to 25 minutes required for withdrawing samples at other points in the processing line. Comparable lots of the raw product for each temperature run was thereby afforded.

The second series, consisting of at least four samples, was taken as the crushed fruit emerged from the crusher-preheater (series B, table 1). These samples were preserved in metaphosphoric acid immediately to serve for subsequent determination of ascorbic acid.

The third series of samples was taken at the filling machine in factory A, and from the tank after heating to 180°F. in factory B (series C, table 1) and consisted of two portions in each case. A fourth series of samples was taken from two cans after the cooling operation (series D, table 1).

[5] Food Machinery Corp., "Super Pulper," 20 tons per hour.

The trimmed tomatoes were sampled and preserved for ascorbic acid analysis at the factory by cutting a slice of approximately 25 gm. from the center of the tomato, and homogenizing with 100 ml. of 2% metaphosphoric acid in 1 N sulfuric acid in a Waring Blendor. Other samples from the processing lines were preserved immediately by pipetting 5 ml. of the juice into 10 ml. of the acid mixture. The preserved samples were analyzed for ascorbic acid either immediately on return to the laboratory or within 24 hours after storage in a cold room. Preliminary investigations had shown that the latter procedure involved no loss of ascorbic acid. Ascorbic acid was determined in duplicate on each filtered extract by the xylene extraction method of Robinson and Stotz ('45). The formaldehyde modification of this method showed that all of the indophenol reducing power of the filtrate was due to ascorbic acid.

The determination of various factors related to the consistency of the juice was made by methods described previously (Kertesz and Loconti, '44). Viscosity of the juice ("gross viscosity") was determined in the Stormer viscosimeter at 30°C. using a 90 ml. sample and 50 gm. driving weight. The serum viscosity was determined at the same temperature in an Ostwald viscosimeter. The calcium pectate content of the serum was determined by the Poore ('34) modification of the Carre and Haynes method ('22), using a preliminary precipitation with 70% ethanol containing 0.05 N hydrochloric acid. The rate of filtration was determined by observing the proportion of 100 ml. of the juice which passed through a 18.5 cm. Whatman no. 12 filter paper in 15 minutes.

RESULTS

Table 1 summarizes the ascorbic acid results and physical characteristics of the juices prepared in factories A and B at the different preheating temperatures. The relation between preheating temperatures and the consistency of the juice was in agreement with previous observations (Kertesz and Loconti, '44). Average figures are recorded for ascorbic acid contents with probable errors for the analyses of whole tomatoes, and ranges for the juice samples. Runs I, II, and III were carried out in plant A on the same day. Variations from 156°–200°F. in the preheating temperatures caused no significant differences in the ascorbic acid content of samples taken at any point in the processing line. A 15 to 19% loss in ascorbic acid was observed in the overall process, nearly all of this occurring during the tomato crushing stage. On another day at plant A, the breaking temperature was lowered to

include juice prepared at 80°F., without added heat. Again there was no relation of the preheating temperature to the ascorbic acid content of the finished tomato juice. In run VII, carried out in plant B, only the "cold break" procedure was used. In spite of differences in the type of breaking and juicing, longer standing in the tanks, and a longer processing time, there was nevertheless approximately the same loss of ascorbic acid during the overall manufacturing procedure. In this plant the greatest loss occurred after the juicing procedure rather than during the breaking operation.

TABLE 1

The relation of preheating temperatures to ascorbic acid losses in the processing line and to consistency characteristics of the juice.

RUN [1]	PREHEATING TEMP.	AVERAGE ASCORBIC ACID (MG./100 GM.) [2]				PROCESSING LOSS	RELATIVE VISCOSITY [3]		CALCIUM + [4] PECTATE IN SERUM	COMPARATIVE FILTRATION RATE
		Series A	Series B	Series C	Series D		Juice (Stormer)	Serum (Ostwald)		
	°F.					%			%	
I	200 (185–203)	31.3 ± 0.65 [5]	27.4	26.0	26.4	15.6	2.20	1.82	.100	6.0
II	170 (168–184)	31.8 ± 0.62	27.3	24.2	25.8	18.8	1.76	1.57	.087	8.0
III	156 (148–176)	31.3 ± 0.69	24.8	26.5	26.8	14.5	1.58	1.32	.038	11.0
IV	185 (170–206)	33.5 ± 0.56	28.2	27.0	27.0	19.4	2.02	1.94	.089	7.0
V	124 (98–146)	Same as IV	28.1	27.6	27.3	18.5	1.58	1.44	000	11.7
VI	80 (76–84)	Same as IV	28.0	27.6	27.5	17.9	1.59	1.45	.000	12.7
VII	80	20.8 ± 0.46	20.7	16.8	16.6	20.2	1.26	1.15	.000	33.0

[1] Runs I–III on same day at plant A, IV–VI on a different day; VII at plant B.

[2] Series A — trimmed tomatoes; series B — after breaking; series C — before can filling; series D — processed juice.

[3] For particulars of the methods see the text.

[4] After preliminary precipitation with acid ethanol.

[5] Probable error of the mean.

The ascorbic acid content of the raw tomatoes was considerably less at plant B than at plant A, resulting in juice with a considerably lower ascorbic acid content. The accompanying paper of Somers, Hamner and Nelson ('45) offers a possible explanation of this finding involving differences in solar illumination prior to harvesting.

In contrast to the negative effect of breaking temperature on the ascorbic acid content of the juice, a distinct effect is noted on consistency characteristics of the juice. Juices prepared by the "cold break" method possessed a low viscosity and high filtration rate. Juice prepared in run VII showed a separation of a watery layer from the juice on standing. No pectic substances could be found in the serum of the juices prepared with preheating temperatures of 124°F. or lower, results which confirm more controlled laboratory experiments (Kertesz and Loconti, '44).

TABLE 2

Effect of heating in an open tank and of hot water processing of canned tomato juice on the ascorbic acid content.

TREATMENT	TIME	ASCORBIC ACID	LOSS IN PROCESSING
	min.	*mg./100 ml.*	%
Juice standing in open tank at 180°F. with constant stirring — Duplicate analyses.	0	15.5	..
	4	15.0	3.2
	10	14.6	5.8
	30	15.0	3.2
	60	13.1	15.5
	90	12.8	17.4
	120	11.1	28.4
Cans processed in boiling water tank. Duplicate cans analyzed 4 days after processing.	0	13.5	..
	10	13.4	0.7
	20	13.3	1.5
	30	13.5	0.0
	60	12.9	4.4
	90	12.4	8.1
	120	12.2	9.6

Since no outstanding differences in the ascorbic acid content of the finished juice could be attributed to variations in the preheating temperatures, the effect of exposure of the juice to air in the heating tank and prolonged processing in the closed can was studied. These experiments were carried out in plant B, where the tomato juice was kept in open 600-gallon stainless steel tanks at 180°F. and continuously stirred. The canned juice (no. 3 size cans) was processed in a tank of boiling water. The results of these experiments are shown in table 2. It is evident that the hot juice exposed to air lost considerable ascorbic acid, amounting to approximately 30% in 2 hours. Excessively long storage of the hot juice in a tank could therefore lead to production of a juice with a considerably lower ascorbic acid content. There was little loss during processing in the sealed can. During the 20 minutes of process-

ing regularly employed at plant B, there was no loss of ascorbic acid; 1 hour of heating resulted in 4% loss, and 2 hours in approximately a 10% loss.

DISCUSSION

The experiments reported show that under efficient manufacturing conditions, tomato juice should contain at least 80% of the ascorbic acid content of the trimmed tomatoes. Wide variations in the preheating temperature, although causing great differences in the consistency, produced no changes in the losses of ascorbic acid during the manufacture of tomato juice. In general, the great stability of ascorbic acid in tomato juice in contrast to its stability in other types of solution is noteworthy. There are apparently no enzyme systems in the juice which are effective in the oxidation of vitamin C; in fact there appears to be present an efficient inhibitor of ascorbic acid oxidation.

Assuming efficient manufacturing conditions, it appears that the greatest single factor in producing juices of widely different vitamin C contents is the amount of vitamin in the tomatoes. The wide variation in market samples of tomato juice (Pressley et al., '44) as contrasted to the smaller variation in tomato juice samples collected at the cannery (Somers, Hamner and Nelson, '45), might be traced to differences in storage conditions as well as to variations in the fruit.

Recently the Council on Foods and Nutrition of the American Medical Association ('45) proposed to place the "seal of acceptance" on canned tomato juice only if this contained in excess of 20 mg. of ascorbic acid per 100 ml. juice. It is clear that in order to obtain freshly made tomato juice which will meet this standard, the tomatoes used in the manufacture must contain an average of at least 25 mg. of ascorbic acid per 100 gm. (trimmed) fruit. In view of inevitable losses of ascorbic acid during storage and distribution, it may also be necessary to specify favorable storage conditions before a "seal of acceptance" can become an effective nutritional measure.

SUMMARY

1. Losses of ascorbic acid were measured during the commercial production of tomato juice, with particular attention to possible variations due to different preheating temperatures.

2. An overall ascorbic acid loss of 15 to 19% occurred during tomato juice manufacture, and this was independent of the preheating temperature. A distinct effect of preheating temperature on consistency characteristics of the juice was evident.

3. The principal loss of ascorbic acid occurred during the "breaking" of the tomatoes. Considerable loss may occur during excessive holding of the hot juice in an open tank, but little during processing in the can.

LITERATURE CITED

CARRE, M. H., AND D. HAYNES 1922 The estimation of pectin as calcium pectate and the application of this method to the determination of the soluble pectin in apples. Biochem. J., vol. 16, p. 60.

COUNCIL ON FOODS AND NUTRITION 1945 Acceptability of foods with high natural vitamin C content. J. Am. Med. Assn., vol. 128, p. 204.

DAGGS, R. G., AND A. G. EATON 1934 Vitamin C content of canned tomato juice. Ind. and Eng. Chem., vol. 26, p. 292.

KERTESZ, Z. I., AND J. D. LOCONTI 1944 Factors determining the consistency of commercial canned tomato juice. New York State Agr. Exp. Sta. Tech. Bull. no. 272.

KOHMAN, E. F., W. H. EDDY AND C. Z. GURIN 1933 Vitamins in canned foods. XIII. Canning tomato juice without vitamin C loss. Ind. and Eng. Chem., vol. 25, p. 682.

POORE, H. D. 1934 Recovery of naringen and pectin from grapefruit residue. Ind. and Eng. Chem., vol. 26, p. 637.

PRESSLEY, A., C. RIDER, M. C. SMITH AND E. CALDWELL 1944 Nutritive value of canned foods. II. Ascorbic acid and carotene or vitamin A content. J. Nutrition, vol. 28, p. 107.

ROBINSON, W. B., AND E. STOTZ 1945 The indophenol-xylene extraction method for ascorbic acid and modifications for interfering substances. J. Biol. Chem., vol. 160, p. 217.

SANBORN, N. H. 1938 Conservation of vitamin C in tomato juice production. The Glass Packer, vol. 17, p. 102.

SOMERS, G. F., K. C. HAMNER AND W. L. NELSON 1945 Field illumination and commercial handling as factors in determining the ascorbic acid content of tomatoes received at the cannery. J. Nutrition, vol. 30, p. 425.

WOKES, F., AND J. G. ORGAN 1943 Oxidizing enzymes and vitamin C in tomatoes. Biochem. J., vol. 37, p. 259.

THE ELIMINATION AND DISTRIBUTION OF SELENIUM IN THE TISSUES IN EXPERIMENTAL SELENIUM POISONING [1]

IRENE ROSENFELD AND O. A. BEATH
Department of Research Chemistry, University of Wyoming, Laramie

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The retention and elimination of selenium from the tissues of livestock raised on seleniferous areas are important from the public health point of view. Smith et al. ('36) found that the rural population of eastern Wyoming, South Dakota, and northern Nebraska excreted from 100 to 200 μg. % of selenium. Normally, human urine in nonseleniferous areas contains no selenium. Smith and his co-workers ('37) reported that the probable source of selenium was of animal origin, such as milk, eggs, and meat as well as cereal grains and vegetables. They reported that the incidence of vague symptoms of ill health, suggestive of gastric and hepatic disorders, were high enough to indicate the probability of cause and effect.

Investigations dealing with the elimination and distribution of selenium on laboratory animals were carried out by Smith et al. ('37, '38) and Anderson and Moxon ('41). Studies dealing with the distribution and elimination of selenium in farm animals are few. Dudley ('36) reported the distribution of selenium in cases of acute and subacute selenium poisoning in livestock. However, he did not study the elimination of selenium in these animals. Since seleniferous areas supply a large amount of our meat, it appeared to us that an investigation on the elimination and distribution of selenium in the tissues of larger animals should merit consideration. Our experiments were designed to study the effect of protein diets upon selenium toxicity, the biochemical changes which occur in selenium poisoning and the distribution and elimination of selenium. The results of the first-mentioned problem were reported elsewhere (Rosenfeld and Beath, '46). In this paper we wish to report on the elimination and distribution of selenium in the different organs.

[1] Approved for publication by the Director of the Wyoming Experiment Station.

EXPERIMENTAL

Yearling ewes raised in Wyoming were used as experimental animals. The ewes were divided into four groups, five animals in each group. Group I received a high protein diet (20% digestible protein); group II was fed with a medium protein diet (12% digestible protein); group III was given a low protein diet (1.5% digestible protein); and group IV, controls, received the same diets without selenium. The percentage composition of the respective diets is given in table 1.

TABLE 1

Percentage composition of diets fed to sheep.

INGREDIENT	HIGH PROTEIN	MEDIUM PROTEIN	LOW PROTEIN
	%	%	%
Ground yellow	30	12.5	
Linseed meal	25	16	
Cottonseed meal	18	10	
Wheat bran	13	12.5	
Molasses	10	20	19
Mineral mixture[1]	4	4	4
Wheat straw		25	25
Dried starch waste			25
Cottonseed hull			25
Corn oil			2

[1] Ground limestone 40%, disodium phosphate 40%, and iodized salt 20%.

The selenium was given by drenching. Water extract of the seleniferous plant, Atriplex canescens, was used as a source of organic selenium. The concentration of selenium in the extract was determined by the method of Klein ('41). However, we found that whether we used organic or the inorganic selenium of sodium selenite (Na_2SeO_3) acute intoxication occurred with the same dose when the selenium was administered orally. Therefore, at the end of the experiment we used the inorganic selenium. Each experimental animal was drenched with 10 mg. of selenium, daily for 21 days, and the dose was increased to 20 mg. daily until the animals developed toxic symptoms. In two intoxicated animals of each group the selenium feeding was discontinued, and in three sheep the dose was increased to 30 mg. daily. The selenium administration was stopped in order to study its elimination from the different tissues and to determine the length of time required for the animals to be free of urinary selenium. Once a week each sheep was placed in a metabolism cage for 24 hours and urine collected with toluol as the preservative. The total quantity of urine excreted was noted and the amount of selenium was calculated on that basis. Blood was taken

from the jugular vein of the animals. Analyses of selenium in the blood, urine and tissues were carried out according to the method of Klein ('41).

DISCUSSION

Urinary excretion of selenium of the three groups of animals which received selenium until death are given in table 2. Groups on high, medium and low protein diets excreted 4.8, 5.5 and 3.5 mg. of selenium, respectively, during 33 days. However, as the administration of selenium was continued, there was a decreased elimination, with subsequent oliguria, and anuria. This decrease was associated with kidney injury as reported by Draize and Beath ('35).

TABLE 2

Excretion of selenium in the urine by the different diet groups. Sheep received selenium until death.

HIGH PROTEIN DIET				MEDIUM PROTIEN DIET				LOW PROTEIN DIET			
Selenium Administered		Excreted		Selenium Administered		Excreted		Selenium Administered		Excreted	
No. days	*mg./ day*	*mg./ day*	*%*	*No. days*	*mg./ day*	*mg./ day*	*%*	*No. days*	*mg./ day*	*mg./ day*	*%*
0	0 (3)[1]		0	0	0 (3)[1]		0	0	0 (3)[1]		0
33	15.0	4.8	31.7	35	15.0	5.5	36.9	29	15.0	3.5	23.1
51	20.0	5.7	28.7	50	20.0	5.7	28.5	40	20.0	1.9	9.6
67	30.0	5.7	19.1	64	30.0	5.3	17.5	48	20.0	2.1	10.5
80	30.0	4.7	15.5	78	30.0	4.7	15.5				
107	30.0	4.7	15.6	108	30.0	2.8	8.3				

[1] Numbers in parentheses indicate the number of animals used.

Table 3 gives the selenium in the urine after selenium feeding was discontinued. Before symptoms of intoxication appeared the high and medium protein diet groups had received in gradual doses a total of 860 mg. and the low protein diet group a total of 360 mg. of selenium. The groups which received the higher quantity of selenium eliminated larger amounts. However, 36 to 38 days after the cessation of selenium administration, all groups eliminated approximately the same amount, and at the end of 50 days all the urines were free or contained only traces of selenium. Smith and his co-workers ('37) reported that cats eliminated the bulk of the selenium within 2 weeks. McConnell ('41), using radioselenium as tagged atoms, studied the distribution and elimination of a single subtoxic dose of selenium in rats and found that from 41 to 43% of the original dose appeared in the urine during the first 24-hour period. We have found that sheep excreted only small amounts of selenium in the first 24 hours. It seems that among different species of

animals there is much variation in the time taken before selenium appears in the urine and time required for its depletion. Therefore, the elimination of selenium in smaller animals can serve only as an approximate guide in the larger animals.

TABLE 3

Elimination of selenium in the urine after selenium feeding discontinued.

	ANIMALS RECEIVING DIETS								
	HIGH PROTEIN			MEDIUM PROTEIN			LOW PROTEIN		
	Selenium			Selenium			Selenium		
	Days	Total amount given	Excreted	Days	Total amount given	Excreted	Days	Total amount given	Excreted
		mg.	*mg./day*		*mg.*	*mg./day*		*mg.*	*mg./day*
Duration of selenium feeding	44	860(2)[1]	5.558	44	860(2)[1]	5.98	44	360(2)[1]	2.85
Selenium was discontinued	8		1.20	8		1.20	13		0.21
Selenium was discontinued	36		0.243	36		0.34	38		0.28
Selenium was distinued	50		Traces	50		Traces	52		0.0
Control		0	0.0		0	0.0		0	0.0

[1] Numbers in parentheses indicate the number of animals used.

Table 4 presents the concentration of selenium in the tissues of animals which received selenium until death and the selenium in the tissues of animals in which the selenium feeding was discontinued for 61 and 64 days. Sheep fed with high and medium protein diets were able to withstand a larger amount of selenium than those on the low protein ration. The increased selenium intake in the higher protein diet groups produced a correspondingly increased storage in the different organs. In all groups the liver and kidney contained the largest amount and the muscle and brain stored the smallest amount of selenium. It is interesting to note that the fat was free of selenium in all groups, regardless of the amount of selenium given. This suggests that a greater deposition of selenium occurred in the organs which contained the higher percentages of protein. Dudley ('36) suggested a replacement of sulfur by selenium in certain amino acids which were utilized in the formation of modified proteins. We found that the livers of animals which died

TABLE 4

Selenium in body tissues. (Parts per million.)

DIET	TOTAL SELENIUM GIVEN		LIVER	KIDNEY	HEART	LUNG	SPLEEN	BRAIN	STOMACH	MUSCLE	FAT	BLOOD
	mg.	*days*										
High protein diet												
1. Se given until death	1922 (3)[1]	116	29.21	3.95	1.75	3.10	3.51	0.8	3.9	0.8	0	3.1
2. Se given until intoxicated then discontinued for 61 days and animal killed	860 (2)	44	1.33	1.47	.99	.48	.69	0	.32	0	0	.42
Medium protein diet												
1. Se given until death	1922 (3)	116	33.17	13.55	9.92	4.91	9.54	..	5.9	0.97	0	1.34
2. Se given until intoxicated then discontinued for 61 days and animal killed	860 (2)	44	1.56	1.06	.26	.67	.71	0	.45	0	0	.61
Low protein diet												
1. Se given until death	632 (3)	64	9.10	6.87	1.90	2.67	1.96	..	..	0.8	..	2.14
2. Se given until intoxicated then discontinued for 64 days and animal killed	360 (2)	44	1.85	1.57	.57	.38	.67	..	..	0	..	.74
Control	0		.75	0.5	0.0	0.2	0.3	..	..	0	..	.59

[1]Numbers in parentheses indicate the number of animals used.

of selenium poisoning showed a decrease in the protein and sulfur content regardless of diet (Rosenfeld and Beath, '46), indicating a possible protein-sulfur-selenium antagonism. In sheep in which the selenium feeding was discontinued for 61 and 64 days the selenium content of the tissues was approximately the same as in the control animals, with the exception of the liver and the kidney. The residual selenium present in the different tissues after discontinuance of selenium feeding was considered negligible since such small quantities of selenium would be too low to produce harmful effects. Smith and his co-workers ('40) reported that an estimated intake of about 1.0 to 1.5 mg. per kilogram per day of inorganic selenium was required to produce important pathologic changes in experimental animals. Our results agree with their observations. Also it can be seen from table 4 that if animals were slaughtered at a time when their selenium intake was high, then the meat obtained can be a source of a large amount of selenium in the human diet. The absence of selenium in the urine can serve as a guide for the selenium depletion in the different organs.

SUMMARY

The elimination and distribution of selenium in sheep receiving graded doses of selenium for 44 and 116 days was studied. Sheep fed with selenium until death eliminated smaller quantities of selenium in the urine toward the end, due to kidney injury, and stored larger amounts in all the tissues.

Selenium was excreted in the urine gradually after selenium administration was discontinued. In 50 days the urine showed only traces or was free of selenium.

The storage of selenium in the different organs depended upon the amount of selenium administered to the animals. In all groups the liver and the kidney contained the largest amount and the brain and muscle the smallest amount of selenium. Other organs showed variations as to the quantity of selenium accumulated. The fat was free of selenium regardless of the selenium intake.

A small amount of selenium was present in all the tissues 61 and 64 days after selenium administration was discontinued. According to our present knowledge this amount was considered insufficient to cause harmful effects.

LITERATURE CITED

ANDERSON, H. D., AND A. L. MOXON 1941 The excretion of selenium by rats on a seleniferous wheat ration. J. Nutrition, vol. 22, p. 103.

DRAIZE, J. H., AND O. A. BEATH 1935 Observations on the pathology of blind staggers and alkali disease. J. Am. Vet. Med. Assn., vol. 86, p. 753.

DUDLEY, H. C. 1936 Toxicology of selenium. I. A study of the distribution of selenium in acute and chronic cases of selenium poisoning. Am. J. Hygiene, vol. 23, p. 169.

KLEIN, A. K. 1941 Report on selenium. J. Am. Off. Agr. Chem., vol. 24, p. 363.

MCCONNELL, K. P. 1941 Distribution and excretion studies in the rat after a single subtoxic subcutaneous injection of sodium selenate containing radioselenium. J. Biol. Chem., vol. 141, p. 427.

ROSENFELD, I., AND O. A. BEATH 1946. I. The influence of protein diets on selenium poisoning. II. Influence of protein diets on selenium poisoning. The chemical changes in the tissues following selenium administration. Am. J. Vet. Research, vol. 7, (in press).

SMITH, M. I., K. W. FRANKE AND B. B. WESTFALL 1936 The selenium problem in relation to public health. U. S. Pub. Health Rep., vol. 51, p. 1496.

SMITH, M. I., E. F. STOHLMAN AND R. D. LILLIE 1940 Studies in chronic selenosis. National Institute of Health, Federal Security Agency, Public Health Service, part 1, Bull. 174.

SMITH, M. I., AND B. B. WESTFALL 1937 Further studies on the selenium problem in relation to public health. U. S. Pub. Health Rep., vol. 52, p. 1375.

SMITH, M. I., B. B. WESTFALL AND E. F. STOHLMAN 1937 The elimination of selenium and its distribution in the tissues. U. S. Pub. Health Rep., vol. 52, p. 1171.

———— 1938 Studies on the fate of selenium in the organism. U. S. Pub. Health Rep., vol. 53, p. 1199.

SECRETION OF VITAMIN D IN MILKS OF WOMEN FED FISH LIVER OIL[1,2]

LOUIS J. POLSKIN, BENJAMIN KRAMER AND ALBERT E. SOBEL
Pediatric Research Laboratory, The Jewish Hospital of Brooklyn, Brooklyn, New York

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The purpose of the experiments reported in this paper was to determine the vitamin D content of human milk following the ingestion of fish oils containing the antirachitic vitamin.

The literature revealed scanty information on the vitamin D content of human milk and the data were difficult to interpret. Of the normal values given in thirteen papers eleven are reported as either no vitamin D or traces only. Van Niekerk ('33) reported 15 to 22 international units (I.U.) per liter. Drummond, Gray and Richardson ('39) reported an average result of 60 I.U. per liter, the range of the values being from 20 to 90. The results in the fortification experiments reported (eighteen papers) are qualitative rather than quantitative and indicate failure to increase the vitamin D content following the administration of fish oils. We therefore planned an experiment in which massive doses were to be supplied.

PLAN OF EXPERIMENT

The mothers were chosen at random from our prenatal clinic; the study was made during the winter of 1941–1942. The diet prescribed was high in protein, low in fat. The carbohydrate recommended was high initially and was gradually decreased. A liberal supply of milk, green vegetables and eggs was part of this diet. The degree of adherence of the mothers to this prenatal diet could not be checked.

Vitamin D in the form of mixed fish liver oil,[3] bioassayed by us, was supplied in a single dose of 32,000 U.S.P. XI units by capsule at each weekly visit to the clinic. Many mothers failed to attend regularly.

[1] These investigations were aided by a grant from Mead Johnson and Company, Evansville, Indiana.

[2] Presented before the meeting of the American Society of Biological Chemists, held in absentia, 1943. Polskin, L. J., B. Kramer and A. E. Sobel, 1943, Secretion of vitamin D in milks of humans fed fish oil. Fed. Proc., vol. 2, p. 68.

[3] The fish liver oil was kindly contributed by Mead Johnson and Company.

During the observation periods, from 32,000 to as much as 480,000 units were consumed. A total of fifty-nine patients were studied in all, twenty-one of whom served as controls who received no vitamin D supplement. Daily milk collections of from ½ to 2½ ounces were made by means of an electrically-driven breast pump during the first 7 to 9 days of lactation. In one case (L. G., no. 55) an 8-day balance study of the amount of the vitamin present in the blood, milk and stools was made.

Bioassay of vitamin D

The rat bioassay method (A.O.A.C., '40) was used to determine the vitamin D content of the milk, eight feedings being made during the first 8 days; the animals were sacrificed at the end of the tenth day. In order to improve the well-being of the test animals, Steenbock's diet no. 2965 was modified by incorporating 3% brewer's yeast [4] at the expense of the yellow corn in the diet. Sobel, Goldfarb and Kramer ('35), Natelson and Sobel ('35) and Bechtel and Hoppert ('36) reported the incorporation of yeast powder for similar reasons. The bones were rated independently by two of us, following essentially the gradations described by Russell, Wilcox, Waddell and Wilson ('34), and then prepared for photographing by the procedure of Taylor, Klein and Russell ('38).

Vitamin D was determined in blood as follows: For the preliminary blood sample 6 ml. of whole blood were mixed with about 35 gm. of the modified no. 2965 diet. This was entirely consumed by the test rat by the seventh day. The basal ration was then supplied until the animals were sacrificed. For bloods obtained following vitamin D supplementation, high vitamin values were anticipated and, therefore, the whole blood was immediately frozen and dehydrated by the vacuum drying method of Cooper and Grabill ('39) as modified by Warkany, Guest and Grabill ('42). Following preliminary test levels, 350 mg. of blood solids were mixed into the 2965 ration as described above for the final bioassay.

Vitamin D was determined in the feces (or soap suds enema) as follows: The fresh collection was immediately mixed with an equal volume of acetone. The material was then extracted with 250–300 ml. portions of diethyl ether for at least four times. The ether solutions were washed thoroughly with water, shaken with anhydrous Na_2SO_4 and allowed to set with the salt over-night. The filtered ether solutions were concentrated under vacuum. The extract of the stool collected before vita-

[4] Mead Johnson.

min D supplementation was begun was dissolved in ether and distributed directly into the basal ration. The residues of the stools following the vitamin D feeding were dissolved in a known amount of corn oil and stored on ice until bioassayed. The oils were fed to the rats orally by the micro syringe. Although this method of concentrating the vitamin D in human feces was not tested critically it is believed that nearly all of the vitamin was extracted.

The vitamin D in each sample was estimated from the average numerical response of rats fed the reference cod liver oil and also from that of a "master curve" which was made. Recourse to the latter was necessary because this method is essentially one of "trial and error" and the supply of milk was limited. In every case we endeavored to use as many test animals as was practical. The milk samples were stored in the cold and preserved with formalin as prescribed. The average numerical values (arbitrary score) for the degree of cure of the rachitic tibiae in fifteen or sixteen test animals per group were determined.[5] Each group received a total of 32 ml. of a 9% reconstituted skim milk solution and a total of 1, 3, 4, 5 or 7 U.S.P. XI units of vitamin D in the form of Reference Cod Liver Oil. A reading of one represents a single, narrow, continuous black-stained line in the decalcified area (metaphysis) of the halved tibia.[5]

Although the colostra were bioassayed independently, the data are not treated separately because the demarcation of colostrum and milk was not too apparent and, moreover, because the total quantity of the first, watery "milk" usually was enough for one to three rats.

RESULTS

Control milks

From a study of twenty-one control mothers[6] vitamin D could not be detected in the whole milk of eighteen cases when as much as 50–60 ml. of milk were bioassayed. The milks of the remaining three mothers showed 17, 28 and 40 I.U. per liter. Most of the bioassays were done on seven to nine rats and occasionally on less, the average number being 7.0. The volumes of milk consumed per rat were from 13 to 68 ml.,

[5]

U. S. P. XI UNITS VITAMIN D SUPPLIED	NUMERICAL RESPONSE FOR GROUP
1	0.4
3	1.4
4	1.9
5	2.1
7	2.3

[6] Four of these patients, nos. 56, 57, 58 and 59, taken from another experiment, received about 1 gm. of lecithin beginning with lactation. The lecithin, bioassayed at a 0.44 gm. level, had no vitamin D.

the average being 38.6. It was difficult to induce the animals to consume larger quantities of milk. Since 1 unit of vitamin D in Reference Cod Liver Oil gave a healing score of 0.4. i.e., theoretically, $\frac{2}{5}$ of a narrow, continuous line of calcification in the stained metaphysis of the split tibia, it is estimated that about 10 I.U. per liter could be detected. It is concluded, therefore, that the vitamin D of control milks was either absent or less than 10 units per liter. Harris and Bunker ('39) pooled 15 gallons of human milk, collected during the winter and estimated that the amount of vitamin D present was about 4 I.U. (and less than 10 I.U.) per liter. Our control values and in fact all those recorded for "normal" values of vitamin D in mother's milk disagree with the high amounts, namely, an average of 60 I.U. per liter, recorded by the English workers Drummond, Gray and Richardson ('39).[7]

Supplementation during pregnancy

Beginning as early as the fifth month of pregnancy and continuing to term, thirty mothers each received a total of from 32,000 to 480,000 I.U. of vitamin D in the form of fish liver oil. The following groups have been arbitrarily arranged: group I, 32,000 to 95,000 I.U.; group II, 128,000 to 224,000; group III, 256,000 to 320,000; group IV, 416,000 to 480,000 I.U. The results are presented in table 1 and summarized in table 2. The striking finding in these studies is the relatively poor increase of vitamin D in the milk in spite of the large amount of the vitamin in the diet. Seven out of twenty-seven patients receiving from 32,000 to as much as 448,000 units secreted no vitamin D in the milks. The mean vitamin D values obtained in the milks were 10 to 62 I.U. per quart.[8] Large variations were observed among these samples. No relationship was apparent between the amount secreted and the total dose supplied except that the average amount of vitamin D in the milks of mothers fed more than 100,000 units (table 1) before term was significantly larger than those fed from 32,000 to 96,000 I.U. It so happens that with this latter group (table 1) also the largest number of days elapsed between the last dose of 32,000 I.U. and the onset of lactation. However, even among the women fed more than 32,000–96,000

[7] Of the twenty-seven patients tested, only three mothers yielded milks which bioassayed 20 to 35 I.U. per liter; twenty-three, from 40 to 90 I.U. per liter and one case (no. 13) had as much as 180 I.U. per liter. These data are particularly high in view of the fact that the authors stated that the dietary was the sole source of vitamin D and calculated to contain from 35 to 220 I.U. of vitamin D daily.

[8] One case (M, no. 44) is omitted from this series. Following a total intake of 416,000 I.U. units, only a single ounce of "colostrum" plus milk, was collected. Bioassayed with only four rats, it was calculated at 625 I.U. per quart. Unfortunately, this patient had poor breast function and it was impractical to obtain further samples.

TABLE 1

The effect of vitamin D administration during pregnancy on the vitamin D content of human milk.

CASE NO.	PERIOD DURING PREGNANCY WHEN VITAMIN D WAS SUPPLIED	TOTAL VITAMIN D FED	DAYS ELAPSED AFTER LAST VITAMIN D INTAKE AND ONSET OF LABOR	VITAMIN D RECOVERED IN MILK		
	Months	U.S.P. XI units (Each dose equals 32,000 units)		Total Rats	Average ml. milk fed to rat	U.S.P. XI units vitamin D per liter of milk
	I. 32,000 to 96,000 units vitamin D administered during pregnancy					
18-P	6½	32,000	71	11	21	31
19-R	7	32,000	59	7	23	0 to trace
20-D	Labor	32,000	1	10	26	91
21-A	Labor	32,000	1	10	28	216
22-K	5½ to 5¾	64,000	98	12	31	0 to trace
23-M	7¼ to 7¾	96,000	36	10	40	40
24-N	6¾ to 7½	96,000	40	6	32	0 to trace
25-L	6½ to 7	96,000	49	7	36	0 to trace
26-G	7 to 7½	96,000	45	5	28	0 to trace
	II. 128,000 to 224,000 units vitamin D administered during pregnancy					
27-L	7¼ to 8¾	128,000	6	11	39	32
28-R	7¾ to 8½	160,000	10	11	36	109
29-S	7½ to 8¼	160,000	21	4	40	25
30-A	5¼ to 7¾	160,000	33	13	30	0 to trace
31-B	7¾ to 8¾	160,000	10	6	28	10
32-M	7¼ to 8½	160,000	15	10	32	45
33-V	7½ to 8¾	160,000	9	5	29	93
34-L	7¼ to labor	192,000 (including 32,000 in labor)	1	5	12	148
35-B	7¼ to 8¾	224,000	6	8	31	140
36-M	7 to 8¾	224,000	8	12	22	26
	III. 256,000 to 320,000 units vitamin D administered during pregnancy					
37-M	7 to 8¾	256,000	10	8	38	53
38-S	6½ to 8¾	256,000	6	10	30	23
39-M	6¾ to 8¾	256,000	8	8	21	61
40-W	5¼ to 8½	288,000	12	13	23	156
41-S	5½ to 8½	320,000	13	6 7	8 32	19
	IV. 416,000 to 480,000 units vitamin D administered during pregnancy					
42-T	6 to 9	416,000	7	9 10	32 8	22
43-B	5¾ to 8½	416,000	17	10	4	138
44-M	5¾ to 8¾	416,000	9	4	8	625
45-A	5½ to 8½	416,000	12	5 7	8 24	42
46-G	5½ to 9	448,000	8	10	8	0 to trace
47-S	5 to 8¾	480,000	7	8	24	92
48-B	4½ to 8	480,000	30	5 3	16 4	24

units there is also no apparent influence of the length of the period between the last dose and beginning of lactation.

When the dose was given precisely at labor, the factor of time is significant with reference to the enrichment of the milk. Thus, case D (no. 20) and case A (no. 21) secreted 91 and 216 units, respectively, of vitamin D per quart of milk following a single dose of 32,000 units during labor. Such levels were not realized in eight mothers who received four to fifteen times this dose during pregnancy, the last dose (32,000 units) being administered as long as 10 days before the onset of labor.

TABLE 2

Summary of experiment.

SUPPLEMENTATION PERIOD OF VITAMIN D	TOTAL CASES	TOTAL INTAKE OF VITAMIN D	VITAMIN D SECRETED PER LITER OF MILK (AVERAGE OF FIRST WEEK'S COLLECTION)			VITAMIN D ABSENT IN MILK
			Lowest value	Highest value	Average	
		U.S.P. XI Units	U.S.P. XI Units			Total cases
0	21 (controls)	Normal diet only	0	40	0 to trace	18
Between 5th and 9th month of pregnancy	7	32,000 to 96,000	0	40	10	5
	9	128,000 to 224,000	0	148	53	1
	5	256,000 to 320,000	19	156	62	0
	6	416,000 to 480,000	0	138	53	1
From 7th month to term	1	192,000 (including 32,000 during labor	.. ..	.. ..	148	..
During Labor only (24 hours)	2	32,000	91	216	153	0
During Labor to first 9 days after delivery	7	320,000 to 400,000	125	583	310	0

Patient L (no. 34), who received a total of 192,000 I.U. of vitamin D between the seventh month to term, including 32,000 units during labor, produced a milk containing 148 I.U. per quart. It is our opinion that the last dose supplied in labor was largely responsible for the quantity recovered.

Post-natal supplementation of vitamin D

Seven patients were given daily doses of 40,000 I.U. of vitamin D by capsule beginning during labor and continuing through the first 7 to 9 days of lactation. The capsule was fed about 6 P. M., and the milk samples were collected about 15 hours later. As seen in table 3 these

TABLE 3

Group V which received 320,000 to 400,000 I.U. of vitamin D beginning with labor and continuing during lactation.

CASE NO.	TOTAL VITAMIN D FED	VITAMIN D RECOVERED IN MILK		
	U.S.P. XI Units (1 dose equals 40,000 units)	Total rats	Total ml. milk fed to rat during bioassay	U.S.P. IX Units vitamin D per liter of milk
49-M	320,000	8	32	125
		11	16	
50-G	320,000	7	32	250
		11	16	
51-W	360,000	9	16	312
52-N	360,000	10	16	156
53-S	360,000	8	16	312
54-B	400,000	9	16	438
55-G	320,000	14	12	583
		9	6	

patients secreted milks containing from 125 to 583 I.U. of vitamin D per quart. The mean value of 310 I.U. corresponded to a mean intake of 348,000 units in the form of fish liver oil. Although the average quantity recovered was low, the mean value is five to six times higher than the means for mothers supplied the vitamin during pregnancy only (table 2).

Balance study with patient G, no. 55

On a 23-year-old negro patient (G, no. 55) of the last group described vitamin D was determined in the milk, blood and stools. Following a daily intake of 40,000 I.U. of vitamin D for 8 days, beginning with

labor, the amount recovered in the milk when bioassayed at 1.5 and 0.75 ml. levels, was 666 and 500 I.U. per quart. Blood samples, taken approximately 14 hours after the dose on the fifth and ninth days, contained 393 and 384 I.U. per 100 ml., respectively, as compared with the 118 I.U. found in the pre-test specimen. (Warkany and Mahon, '40, bioassayed sera of 155 normal humans and reported a range from 66 to 165 I.U. per 100 ml.) The pre-test stool contained no vitamin D; those collected as quantitatively as practical until the end of the ninth day, totalled 22,820 I.U. of vitamin D. The data are summarized in table 4.

We observed the following recovery values of the ingested vitamin D for case G (no. 55):

TABLE 4

Balance study of vitamin D in case no. 55 (secundipara; colored; 60 kg.; age 23 years).

POST-PARTUM DATE	GIVEN DAILY BY CAPSULE 40,000 U.S.P. XI UNITS VITAMIN D	U.S.P. XI UNITS VITAMIN D RECOVERED					
		Milk		Blood		Stool	
		Units per liter	Rats tested	Units per liter	Rats tested	Units per pooled output	Rats tested
9/23/42 A. M.	(labor)-0			1180	3	0	4
9/23 6 P. M.	40,000						
9/24	40,000					2900	10
9/25	40,000						
9/26	40,000					4250	10
9/27	40,000	666	14	3930 [1]	12		
9/28	40,000	and				10310	10
9/29	40,000	583	9	(9/27/42)			
9/30	40,000			3850 [1]	12	4820	11
10/1	0			(10/1/42)			

[1] About 14 hours after capsule was given.

(a) In milk, 1.3%, assuming 1 quart is secreted in 24 hours and deducting the amount given by a theoretical pre-test milk having a liberal level of 50 I.U. per quart.

(b) In stools, 6.9%, assuming that this is the total which was not absorbed, that no changes (due to bacterial effects) occurred in passage through the tract, and that the blood contributed none of this amount.

(c) In the blood about 14 hours after the dose, 36.5%; assuming 90 ml. of blood per kilogram body weight (Best and Taylor, '39) and deducting the quantity found in the pre-test sample.

From these values, it is obvious that a large part of the ingested vitamin D is unaccounted for.

DISCUSSION OF RESULTS

After reviewing papers reporting tests where mothers were fed vitamin D in the form of fish liver oil during pregnancy [9] it was concluded that perhaps larger amounts than those fed are necessary to increase the content of vitamin D in human milk. We supplied, therefore, significantly higher levels of the vitamin during pregnancy, and were not successful in seven out of twenty-seven cases. Milks were enriched with vitamin D in every case when doses of 32,000 to 40,000 I.U. were given during labor or thereafter. As early as 1924, Hess and Weinstock observed rickets in fifteen out of twenty-eight babies born of mothers who received a total of nearly 16 ounces (estimated to be about 37,000 I.U.) of cod liver oil in the eighth and ninth months of pregnancy. Moreover, when 1000 I.U. of vitamin D as halibut liver oil were supplied daily to six English mothers during the latter half of pregnancy, the subsequent milks were not appreciably richer in the factor than those of control subjects (Drummond, Gray and Richardson, '39). Apparently, the capacity to transfer the vitamin D of fish liver oil to breast milk is quite limited. Nevertheless, the possible beneficial effects on both mother and fetus, e.g., improvement in the Ca and P retention, should not be overlooked (Macy et al., '30; Liu and coworkers, '41). In our study with patient G (no. 55) who ingested 40,000 I.U. of the vitamin daily beginning with the onset of labor, it was found that approximately 1.3% of the ingested vitamin was accounted for in the milk; and in two patients (D, no. 20 and A, no. 21), fed a single dose of 32,000 I.U. during labor only, about 0.3 and 0.7% was similarly secreted.

Seven references were found in the literature to studies in which attempts were made to increase the vitamin D level of cows' milk by feeding fish liver oil specifically.

The early workers (Lesne and Vagliano, '24; Wagner and Wimberger, '25; Golding and his coworkers, '26, '28; Both, '34) did succeed in increasing somewhat (units omitted) the vitamin D of cow's milk by feeding cod liver oil. Steenbock et al. ('30) supplied a cow daily for over 2 years, 180 gm. of cod liver oil (about 9700 I.U.) and found that the antirachitic properties of her milk was not improved over that yielded by control cows. By employing daily massive doses, Krauss, Bethke and Wilder ('33) were more successful in raising the vitamin D content of the butterfat. We calculated from their data a recovery of about 0.8 to 1.6% of the ingested vitamin D in the milk.

[9] A bibliography will be submitted on request.

It is apparent that vitamin D must be available beyond the normal intakes just prior to lactation in order to enrich the milk with the factor. Eufinger, Wiesbader and Focsaneanu ('29) concluded that the transfer of vitamin D to the milk is an immediate one because rachitic rats were healed when fed milk from mothers given 10 drops of vigantol 1 day only before lactation. These investigators found that the milk from mothers fed 10 drops vigantol (irradiated ergosterol in oil) several months before delivery also prevented rickets in rats, in contrast to colostrum or milk from control mothers fed ad libitum in the prophylactic bioassay. Unfortunately, these milks were not bioassayed quantitatively. McCollum et al. ('27) stated that young rats were afforded a much higher degree of protection against rickets when the cod liver oil was supplied to the mother during lactation only than during pregnancy only. Other attempts to fortify human breast milk by supplying the vitamin during lactation are noteworthy. Gerstenberger and Russell ('30) were not successful in curing rachitic infants with the milk from lactating mothers fed 15 ml. (about 1170 I.U.) of cod liver oil daily (test period not stated), nor with the milk from those on an already adequate diet further supplemented daily with 1 tablespoonful of cod liver oil (250 I.U.) for 45 days (Gerstenberger, Hartman and Smith, '27). Weech ('27) concluded from his studies that a certain amount of the antirachitic vitamin does pass into the mother's milk because the degree of rickets in his series of babies was, in general, inversely proportional to the previous intake of oil by the nursing subject. Wide variations were encountered, however.

The fate of vitamin D in the body

Our knowledge concerning the fate of vitamin D in the body is still unsatisfactory. On the assumption that the amount of vitamin D which appeared in the feces of patient G (no. 55) represented that quantity (7%) which escaped absorption, it is concluded that the mixed fish liver oil was absorbed very efficiently. Only three papers could be found in the literature reporting bioassays of human feces for vitamin D. Hess, Weinstock and Gross ('33) and Hess ('33) bioassayed stools of infants fed viosterol, cod liver oil, and "vitamin D" milks and concluded that the amount of vitamin D excreted was dependent on the intake, absolute units were not reported. Windorfer ('38) recovered 3 to 14% (average 7%) of the vitamin D fed to rachitic infants in these fecal extracts. Using dogs, Windorfer recovered from 1 to 29% (average 20% of the ingested vitamin D after 48 hours.

Heymann ('37, '38) supplied adult dogs with a single dose of 250,000 U.S.P. units of vitamin D in the form of viosterol in oil or crystalline D_2 in propylene glycol and was able to find appreciable quantities excreted in the stool 6 to 8 months after the supplement was given. Morgan and Shimotori ('43) using essentially the Heymann ('37) method found little vitamin D on the first day and none thereafter, in the stools of three young dogs (50–85 kg.) which were fed a single massive dose of 20,000 I.U. of vitamin D per kilogram body weight in the form of tuna liver oil, irradiated ergosterol and delsterol (i.e., irradiated cholesterol).

It appears from these studies that man and dog absorb vitamin D very well. However, from studies with the chick (Klein and Russell, '31) and the cow (Hart et al., '29, '30; Hess et al., '32; Krauss, Bethke and Wilder, '34) it is agreed that a relatively huge amount of the ingested vitamin D appears in the feces. It is interesting to point out, moreover, that the associate fat soluble vitamin, namely, vitamin A, is absorbed to the extent of 90% in the hen (Russell et al., '42), rat (Vedder and Rosenberg, '38; De, '37; Baumann and coworkers, '34; Davies and Moore, '34), cat (Ahmad, '31), infant and adult humans maintained on various diets (Rowntree, '30; Anderson, '38, '39; Wilson, Das Gupta and Ahmad, '37; Catel, '38).

Although the urine of patient G (no. 55) was not bioassayed for vitamin D, it is of interest that Hess et al. ('33) found no antirachitic activity in 50 ml. of urine from a cow fed daily 270,000 I.U. of vitamin D as irradiated yeast. Moreover, Lawrie and his coworkers ('41) found no vitamin D in the urine of a normal human (sex not stated) 24 hours following a dose of 9000 I.U. in the form of calciferol, nor in the urine of a dog fed 20,000 I.U. similarly. No vitamin A was recovered in the urines of pregnant women, nor in two given 12,000 I.U. of vitamin A (as halibut oil) for 1 month or 70,000 I.U. daily for 1 week. These values for the vitamin A in urines of pregnant women are much less than those reported by Gaehtgens ('37) for normal pregnant human subjects.

The failure to find appreciable amounts of vitamin D in the milks of mothers fed this supplement prior to labor may be due to the inactivation of this factor. It is agreed in the literature that the larger proportion of the ingested vitamin D is presumably destroyed or converted to some inactive material. Light, Wilson and Frey ('34) concluded that vitamin D, unlike vitamin A, is not stored in the liver of this species since "livers of cows which for months have been receiving approximately 70,000 units (189,000 I.U.) of vitamin D in excess of the amount secreted in the milk or excreted in the feces have only one unit (2.7 I.U.)

of vitamin D per gram of fresh liver at the end of this time." Guerrant et al. ('38) fed calves cod liver oil and irradiated yeast for as long as 7 months and are in agreement with the above workers. Heymann ('37) using rabbits found that various tissues retained vitamin D for several weeks following a single dose of 200,000 U.S.P. units of viosterol in oil. Likewise, Morgan and Shimotori ('43) fed dogs 200,000 I.U. per kilogram body weight accounted for less than 10% of the ingested vitamin D in the tissues. Similar findings were reported by Remp ('41) using rachitic rats. Following a single dose of 100 I.U. of crystalline vitamin D_2, from $\frac{1}{3}$ to $\frac{1}{4}$ of the amount administered remained in the body of the rat at the end of 24 hours. After 10 days, only 5 to 10% was found in the body. Small and relatively constant amounts of the vitamin (quantity not stated) were observed in the feces during these periods.

Studies of vitamin D retention in human tissues have been done on only four children to date, these being given large amounts of vitamin D from 36 hours to 26 days before exitus (Vollmer, '39, '41; and Windorfer, '38). In spite of the massive quantities (over a million units) supplied by Vollmer to three of these cases by intramuscular injection, the recovery of the vitamin in the tissues studied was inappreciable. Windorfer ('38) fed 15 mg. (600,000 I.U.) of vitamin D_2 (irradiated ergosterol) to a baby with pneumonia 2 weeks before death and found only 3.3% in the brain, 4% in the kidney, and traces in liver and intestinal tissues using the colorimetric procedure of Brockman.

Although it is known that the concentration of vitamin D in the blood stream governs the amount secreted in the milk (cow's), the processes involved are a matter of speculation. Thus far, for humans, we do not know the limits of this secretion, nor the minimal amount of a given source of the vitamin which will just raise the vitamin D level of human milk.

That the medium containing the vitamin plays a significant role in the effectiveness of vitamin D has been well established (Lewis, '35, '36; Greaves and Schmidt, ''34; Shelling, '37; Supplee et al. '36). Hess, Lewis, McLeod and Thomas ('31) found that irradiated yeast induced a more potent milk per unit of vitamin D fed than viosterol. Russell et al. ('34) successfully increased the vitamin D level of cow's milk beyond that incurred by the feeding of irradiated ergosterol in oil when 0.75% hydroquinone was added. Thus the problem of increasing the vitamin D content of the milk as secreted still remains an open field for further study.

SUMMARY

1. The vitamin D level in the milks of eighteen mothers who received no preformed sources of the factor in the dietary during pregnancy was inappreciable, ranging from traces to less than 10 I.U. per quart. Three cases showed values of 17, 28 and 40 I.U. per quart.

2. The average vitamin D content per quart of milk of mother supplied during the latter half of pregnancy was as follows:

(a) 10 I.U. for seven mothers given from 32,000 to 96,000 I.U. of supplement.

(b) 53 I. U. for nine mothers given from 128,000 to 224,000 I.U. of supplement.

(c) 62 I.U. for five mothers given from 256,000 to 320,000 I.U. of supplement.

(d) 53 I.U. for six mothers given from 416,000 to 480,000 I.U. of supplement.

3. Two mothers given a single dose of 32,000 I.U. during labor secreted milks from 3 to 8 days later, which bioassayed 91 and 216 I.U. per quart (0.3 and 0.7%, respectively, of calculated recovery). Seven women supplied 40,000 I.U. of vitamin D daily from labor to about 10 days later yielded milks, the vitamin D content of which averaged 310 I.U. per quart (range 125 to 583 I.U.).

4. It is concluded, therefore, that for the purpose of fortifying human milk with vitamin D for infant use, supplementation of the mother with fish liver oil during pregnancy is not a practical method. Other beneficial effects on mother and fetus are not overlooked, however. Vitamin D must be taken just prior to or during lactation in order to enrich the milk with the factor appreciably.

5. The minimal dose of vitamin D which, when added as a supplement to the mother's basal diet, will yield a satisfactory increase of vitamin D in her milk has not been determined.

6. In an 8-day balance study a patient who received 40,000 I.U. daily in the latter group secreted about 1.3% of the ingested vitamin D in the milk and 6.9% in the stools. Approximately 36% of the factor was accounted for in blood samples taken about 14 hours after the dose. It is presumed that the vitamin D of fish liver oil was very efficiently absorbed, the larger proportion, however, having been inactivated or destroyed very rapidly.

LITERATURE CITED

AHMAD, B. 1931 The fate of carotene after absorption in the animal organism. Biochem. J., vol. 25, p. 1195.

ANDERSEN, O. 1938 The effect of the administration of paraffin oil on the resorption of vitamin A in man. Hospitalstid. (Supp., Festskr. Bisp. Hosp.), vol. 81, p. 29.

———— 1939 Untersuchungen über die Wirkung von Paraffinöleingabe auf die Resorption des A-Vitamins bei Menschen. Klin. Wochenschr., Bd. 18, p. 499.

BAUMANN, C. A., B. M. RIISING AND H. STEENBOCK 1934 The absorption and storage of vitamin A in the rat. J. Biol. Chem., vol. 107, p. 705.

BECHTEL, H. E., AND C. A. HOPPERT 1936 A study of the seasonal variation of vitamin D in normal cow's milk. J. Nutrition, vol. 11, p. 537.

BEST, C. H., AND N. B. TAYLOR 1939 Physiological basis of medical practice. Second edition. Williams and Wilkins Co., Baltimore, Md.

BOTH. R. G. 1934 Ph. D. Thesis. Cited by A. T. R. Mattick, 1937: "The handling of milk and milk products." Ministry of Agriculture and Fisheries, London, Bull. no. 37, p. 97.

CATEL, W. 1938 Klinische und tierexperimentelle Studien über die normale und pathologische Physiologie des A-Vitamins. Monatschr. für Kinderh., Bd. 73, S. 316.

COOPER, M. L., AND F. J. GRABILL 1939 A simplified apparatus for the preservation of bacterial cultures in the dried state. J. Lab. Clin. Med., vol. 25, p. 184.

DAVIES, A. W., AND T. MOORE 1934 Distribution of vitamin A in organs of normal and hypervitaminotic rat. Biochem. J., vol. 28, p. 288.

DE, N. K. 1937 Assimilation of vitamin A and carotene by rats from some common foods with note on conversion factor I.V./E. proposed by International Vitamin Conference. Indian J. Med. Research, vol. 24, p. 751.

DRUMMOND, J. C., C. H. GRAY AND N. E. G. RICHARDSON 1939 The antirachitic value of human milk. Brit. Med. J., vol. 2, p. 757.

EUFINGER, H., H. WIESBADER AND L. FOCSANEANU 1929 Tierexperimentelle Studien zur Vigantolprophylaxe. Klin. Wochenschr., Bd. 8, S. 826.

GAEHTGENS, G. 1937 Über die Ausscheidung von Vitamin A in der Gravidität. Klin. Wochenschr., Bd. 16, S. 52.

GERSTENBERGER, H. J., J. I. HARTMAN AND D. N. SMITH 1927 The antirachitic value of human milk. Calif. and Western Med., vol. 27, p. 40.

GERSTENBERGER, H. J., AND G. R. RUSSELL 1930 Studies on wet nurses receiving irradiated ergosterol. Acta Paediat., vol. 11, p. 32.

GREAVES, J. D., AND C. L. A. SCHMIDT 1934 The action of viosterol in jaundiced rachitic rats. Univ. of Calif. Publ., Physiol., no. 6, vol. 8, p. 49.

GUERRANT, N. B., R. A. MORCK, S. I. BECHDEL AND N. W. HILSTON 1938 The storage of vitamin D in the tissues of growing calves. Proc. Soc. Exp. Biol. Med., vol. 38, p. 827.

HARRIS, R. S., AND J. W. M. BUNKER 1939 The vitamin D potency of human breast milk. Am. J. Pub. Health, vol. 29, p. 744.

HART, E. B., H. STEENBOCK, O. L. KLINE AND G. C. HUMPHREY 1930 Dietary factors influencing calcium assimilation. XIII. The influence of irradiated yeast on the calcium and phosphorus metabolism of milking cows. J. Biol. Chem., vol. 86, p. 145.

HART, E. B., H. STEENBOCK, E. C. TEUT AND G. C. HUMPHREY 1929 Dietary factors influencing calcium assimilation. The influence of cod-liver oil upon the calcium metabolism of milking cows. J. Biol. Chem., vol. 84, p. 359.

HESS, A. F. 1933 The fate of various antirachitics within the body. Acta Paediat., vol. 16, p. 467.

HESS, A. F., J. M. LEWIS, F. L. MACLEOD AND B. H. THOMAS 1931 Antirachitic potency of the milk of cows fed irradiated yeast or ergosterol. A clinical test. J. Am. Med. Assn., vol. 97, p. 370.

HESS, A. F., AND M. WEINSTOCK 1924 Rickets as influenced by the diet of the mother during pregnancy and lactation. J. Am. Med. Assn., vol. 83, p. 1558.

HESS, A. F., R. F. LIGHT, C. N. FREY AND J. GROSS 1932 A study of the milk, blood, and excreta of cows fed moderate and excessive amounts of irradiated yeast or ergosterol. J. Biol. Chem., vol. 97, p. 369.

HESS, A. F., M. WEINSTOCK AND J. GROSS 1933 A biologic assay of blood and feces of infants receiving various antirachitics. Proc. Soc. Exp. Biol. Med., vol. 30, p. 1357.

HEYMANN, W. 1937 Metabolism and mode of action of vitamin D. II. J. Biol. Chem., vol. 118, p. 371.

———— 1937–38 Metabolism and mode of action of vitamin D. IV. J. Biol. Chem., vol. 122, p. 249.

KLEIN, D., AND W. C. RUSSELL 1931 The fate of the antirachitic factor in the chicken. The antirachitic factor balance in the growing chick. J. Biol. Chem., vol. 93, p. 693.

KRAUSS, W. E., R. M. BETHKE AND W. WILDER 1933 The effect of feeding a cod liver oil concentrate to cows on the vitamin D content of milk. J. Dairy Sci., vol. 16, p. 549.

———— 1934 The relative efficiencies of irradiated ergosterol and irradiated yeast for the production of vitamin D milk. J. Dairy Sci., vol. 17, p. 685.

LAWRIE, N. R., T. MOORE AND K. J. RAJAGOPAL 1941 The excretion of vitamin A in urine. Biochem. J., vol. 35, p. 825.

LESNE, E., AND M. VAGLIANO 1924 De l'influence du lait de femme sur le rachitisme experimental. Compt. Rend. Soc. Biol., vol. 91, p. 143.

LEWIS, J. M. 1935 Clinical experience with crystalline vitamin D: The influence of menstruum on the effectiveness of the antirachitic factor. J. Pediat., vol. 6, p. 362.

———— 1936 Further observations on the comparative antirachitic value of crystalline vitamin D administered in milk, corn oil, or in propylene glycol. J. Pediat., vol. 8, p. 308.

LIGHT, R. F., L. T. WILSON AND C. N. FREY 1934 Vitamin D in blood and milk of cows fed irradiated yeast. J. Nutrition, vol. 8, p. 105.

LIU, S. H., H. I. CHU, C. C. SU, T. F. YU AND T. Y. CHENG 1940 Calcium and phosphorus metabolism in osteomalacia IX. Metabolic behavior of infants fed on breast milk from mothers showing various states of vitamin D nutrition. J. Clin. Inv., vol. 19, p. 327.

MCCOLLUM, E .V., N. SIMMONDS, J. E. BECKER AND P. G. SHIPLEY 1927 Studies on experimental rickets. XXVIII. Does vitamin D pass into the milk? Am. J. Dis. Child., vol. 33, p. 230.

MACY, I. G., H. A. HUNSCHER, B. NIMS AND S. S. MCCOSH 1930 Metabolism of women during reproductive cycle. Calcium, phosphorus, and nitrogen utilization in lactation before and after supplementing the usual home diets with cod liver oil and yeast. J. Biol. Chem., vol. 86, p. 59.

MORGAN, A. F., AND N. SHIMOTORI 1943 The absorption and retention by dogs of single massive doses of various forms of vitamin D. J. Biol. Chem., vol. 147, p. 189.

NATELSON, S., AND A. E. SOBEL 1935 A new method for the separation of sterols from vitamin D-containing materials. J. Biol. Chem., vol. 109, p. 687.

OFFICIAL AND TENTATIVE METHODS OF ANALYSIS OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS 1940 The Collegiate Press, George Banta Publishing Company. Menasha, Wisconsin.

REMP, D. G. 1941 Storage and excretion of vitamin D in the rat after a single oral dose. J. Biol. Chem., vol. 140, Scientific Proceedings, Soc. of Biol. Chemists, p. CV.

ROWNTREE, J. I. 1930 A study of the absorption and retention of vitamin A in young children. J. Nutrition, vol. 3, p. 265.

RUSSELL, W. C., M. W. TAYLOR, H. A. WALKER AND L. J. POLSKIN 1942 The absorption and retention of carotene and vitamin A by hens on normal and low fat rations. J. Nutrition, vol. 24, p. 199.

RUSSELL, W. C., D. E. WILCOX. J. WADDELL AND L. T. WILSON 1934 The relative value of irradiated yeast and irradiated ergosterol in the production of vitamin D milk. J. Dairy Sci., vol. 17, p. 445.

SHELLING, D. H. 1937 Calcium and phosphorus studies. The effect of emulsification on the potency of viosterol in the treatment of rickets in children. J. Pediat., vol. 10, p. 748.

SOBEL, A. E., A. R. GOLDFARB AND B. KRAMER 1935 Studies of incurable rickets. II. Role of the "local factor" and of viosterol in the pathogenesis of rickets due to beryllium. J. Biol. Chem., vol. 108, p. 395.

STEENBOCK, H., E. B. HART, F. HANNING AND G. C. HUMPHREY 1930 Fat-soluble vitamins. The anti-rachitic value of cow's milk as modified by feeding of irradiated yeast. J. Biol. Chem., vol. 88, p. 197.

SUPPLEE, G. C., S. ANSBACHER, R. C. BENDER AND G. E. FLANIGAN 1936 The influence of milk constituents on the effectiveness of vitamin D. J. Biol. Chem., vol. 114, p. 95.

TAYLOR, M. W., D. KLEIN AND W. C. RUSSELL 1938 Photographing line tests in vitamin D assays. Ind. Eng. Chem., Anal. Ed., vol. 10, p. 26.

VAN NIEKERK, J. 1933 Quantitative data on the content of antirachitic vitamin in cow's milk and mother's milk. Maandschr. v. Kindergeneesk., vol. 2, p. 525.

VEDDER, E. B., AND C. ROSENBERG 1938 Concerning toxicity of vitamin A. J. Nutrition, vol. 16, p. 57.

VOLLMER, H. 1939 Distribution of vitamin D in the body after administration of massive doses. Am. J. Dis. Child., vol. 57, p. 343.

———— 1941 Distribution of vitamin D in brain after repeated administration of massive doses; histologic investigation of D-hypervitaminosis. Arch. Pediat., vol. 58, p. 9.

WAGNER, R., AND H. WIMBERGER 1925 Über den Einfluss von Lebertranverfutterung. Zeit. für Kinderheilk. Bd. 40, S. 295.

WARKANY, J., G. M. GUEST AND F. J. GRABILL 1942 Estimation of vitamin D in blood serum III. Vitamin D in human serum during and after periods of ingestion of large doses of vitamin D. J. Lab. and Clin. Med., vol. 27, p. 557.

WARKANY, J., AND H. E. MAHON 1940 Estimation of vitamin D in blood serum II. Level of vitamin D in human blood serums. Am. J. Dis. Child., vol. 60, p. 606.

WEECH, A. A. 1927 The influence of the administration of cod liver oil to the mother on the development of rickets in the infant. Bull. Johns Hopkins Hosp., vol. 40, p. 244.

WILSON, H. E. C., S. M. DAS GUPTA AND B. AHMAD 1937 Studies on the absorption of carotene and vitamin A in human subjects. Indian J. Med. Research, vol. 24, p. 807.

WINDORFER, A. 1938 Über die Vitamin-D-Resorption bei Vera-Breichung hoher dosen (Vitamin D-Stoss). Klin. Wochenschr., Bd. 17, S. 228.

VITAMIN A REQUIREMENTS IN CALVES[1]

J. M. LEWIS AND LOGAN T. WILSON

Department of Pediatrics, New York University College of Medicine; Department of Laboratories, Beth Israel Hospital, New York City and The Walker Gordon Laboratories, Plainsboro, New Jersey

TWO FIGURES

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In previous communications (Lewis et al., '39, '42) we have reported that the minimum vitamin A requirements in infants are of the same order of magnitude as in rats, namely, 20 to 35 U.S.P. units per kilogram of body weight. This level of vitamin A intake is adequate for fairly good growth and prevents the development of xeropthalmia and other gross manifestations of vitamin A deficiency. However, in order to obtain optimal blood levels of vitamin A and good storage in the liver, the requirements for vitamin A are increased several fold.

The present study was undertaken to obtain information on the relationship of vitamin A intake to growth, blood concentration of vitamin A and liver storage in young calves.

PLAN OF EXPERIMENT

Twenty-six Grade Holstein calves, male and female, 2 to 9 days old were placed on a diet nearly devoid of vitamin A in order to deplete their stores of this vitamin. At first they were fed skimmed milk and when they were able to ingest solid food, they were given the following ration: ground barley 27 parts, linseed oil meal 23 parts, wheat middlings 11.5 parts, oat mill feed 40 parts, ground limestone 3 parts, salt 0.5 parts and irradiated yeast 0.1 parts. This low vitamin A ration is a modification of the one described by Boyer and his co-workers ('42). Skimmed milk was discontinued when the calves were 16 weeks of age. The calves were housed in a separate building and were allowed to run in dry lots when the weather was favorable.

The animals were weighed twice a week and when a cessation in gain in weight occurred they were divided into six groups, each group consisting of four calves. Thirty-two, 64, 128, 256, 512 and 1024 U.S.P. units

[1] This study was aided by a grant from the Borden Company.

of vitamin A per kilogram body weight were fed to each of these groups, respectively. The daily dose of vitamin A was adjusted weekly to the weight of each calf and was given in a capsule. The vitamin A supplement was obtained from shark liver oil and contained 50,000 units of vitamin A per gram. The animals on the lowest vitamin intake were tested for night blindness throughout the entire experiment.

Blood samples for vitamin A determination were taken from each calf at the end of the depletion period and at approximately monthly intervals while receiving the vitamin A supplement.

Two calves in each group received the supplement for 4 and 8 months, respectively, and were then sacrificed. Vitamin A analyses were carried out on the livers and histological examination of the various organs were made on those animals receiving the smallest amounts of vitamin A in order to determine whether there were any histological evidences of vitamin A deficiency.

The methods of determining the vitamin A in blood and liver were based on the Carr-Price reaction and have been the same as were used in previous investigations (Lewis et al., '39, '42).

For the most part, the calves appeared healthy and continued to grow after the vitamin A supplement feeding was started. The chief difficulty experienced was a low grade type of pneumonia, which a number of the calves contracted while being depleted. The first two calves to show signs of depletion died of pneumonia. Two calves started in group I died after 2 and 3 weeks on supplement, and one in group V after 2 weeks on supplement. All of these calves were replaced and their records do not show in the data reported. Four calves, no. 415, group I; no. 416, group II; no. 427, group III and no. 431, group V, showed some evidence of pneumonia but survived after treatment with sulfapyradine. Calf no. 431 was treated for pneumonia on three different occasions, and it is questionable whether the data on this calf should be included in the averages for the group. For this reason, the data on calf no. 431 were eliminated in the computation of the averages of the gain in weight and blood levels in group V. Of the twenty four calves which were killed at the completion of their feeding period, thirteen showed some post-mortem evidence of having had pneumonia. Some of the calves developed diarrhea during the depletion but this symptom disappeared quite promptly after vitamin A supplement was given.

RESULTS

A comparison of the rate of growth of the various groups of calves may be observed in figure 1. It will be noted that there was no signifi-

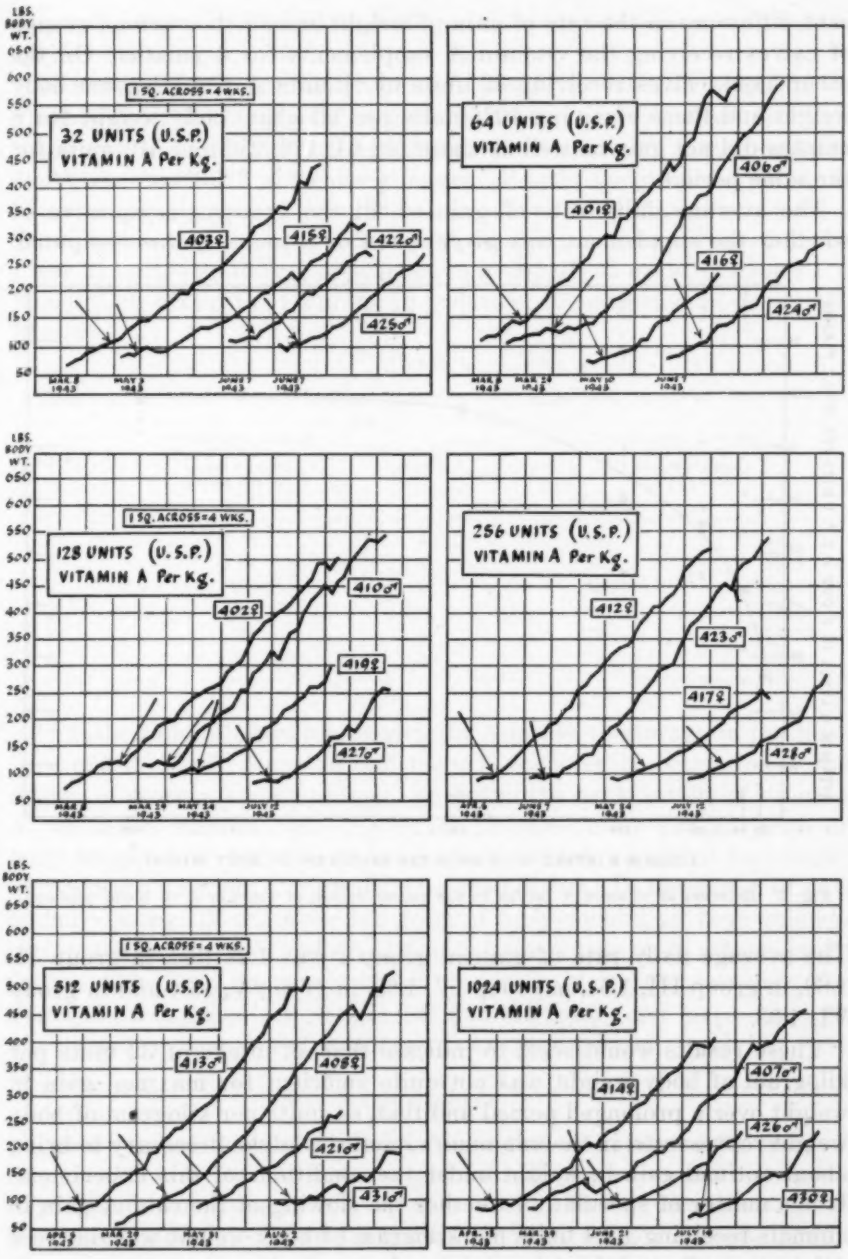

Fig. 1 Growth curves of groups of calves fed various amounts of vitamin A.

cant difference in the rate of gain of weight among the various groups of calves receiving the vitamin A supplements for 4 months. On the other hand, calves receiving 32 units of vitamin A per kilogram body weight and those receiving 1024 units per kilogram body weight for 8 months did not gain as well as those fed 64, 128, 256 and 512 units for the same period.

The average daily rate of gain of all the groups, irrespective of whether the supplement was given for 4 or 8 months, was computed.

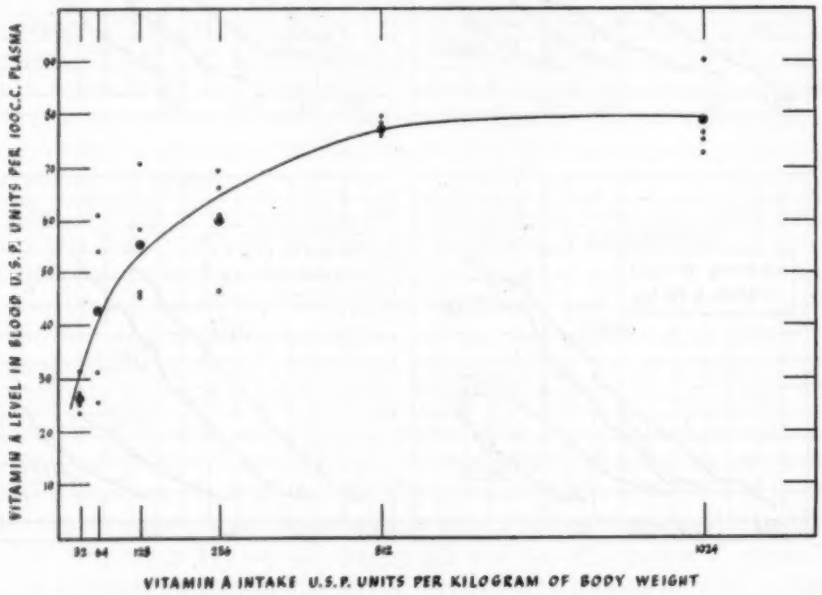

Fig. 2 Relation of vitamin A intake to the concentration of vitamin A in blood plasma.

The average daily rate of gain in group I was 1.23 lbs., in group II, 1.60, in group III, 1.54, in group IV, 1.60, in group V, 1.64 and in group VI, 1.33.

These results would seem to indicate that an intake of 32 units per kilogram of body weight was not quite sufficient for maximal gain in weight over a prolonged period and that, 64 units per kilogram of body weight represented in the minimum vitamin A intake necessary to bring about optimal gain in weight under the conditions of this experiment. It is a matter of speculation whether the slowing of the rate of gain in animals receiving 1024 units per kilogram of body weight was due to a deleterious effect of this large intake of vitamin A on the rate of growth

or was merely coincidental. Additional data would be required to clear up this question.

The relationship of the vitamin A intake to the concentration of vitamin A in the blood is shown in figure 2. It will be observed that there was a direct correlation between the vitamin A intake and the blood level until 512 units per kilogram of body weight were given, at which intake, maximal concentrations of vitamin A were obtained. Thus, (table 1) the average vitamin A concentration in calves of group I was 27 units per 100 ml. of plasma, in group II, 42 units, in group III, 55 units, in group IV, 60 units, in group V, 77 units and in group VI, 78 units.

TABLE 1

Vitamin A concentration in the blood of calves receiving various intakes of vitamin A.

GROUP	VITAMIN A INTAKE PER KILOGRAM BODY WEIGHT (U.S.P. UNITS)	NO. OF CALVES	VITAMIN A LEVEL PER 100 ML. BLOOD PLASMA (U.S.P. UNITS)	
			Average	Range
1	32	4	27	24–27
2	64	4	42	26–61
3	128	4	55	45–71
4	256	4	60	46–69
5	512	3	77	76–79
6	1,024	4	78	73–90

In table 2 the relationship of vitamin A intake to storage in the liver is recorded. It will be noted that there is considerable variation in the degree of storage among animals receiving the same intake of vitamin A. Of seven animals receiving either 32 or 64 units per kilogram of body weight four had no storage and the remaining three had slight to moderate amounts of vitamin A in their livers. In the group of animals receiving 128 units per kilogram of body weight, all four animals had some storage of vitamin A although the amounts were rather small. On the other hand, moderate stores of vitamin A were observed in the livers of most animals receiving 256 and 512 units, and large amounts of vitamin A were found in three of the four animals receiving 1024 units.

DISCUSSION

The results of this experiment indicate that an intake of 32 units of vitamin A per kilogram body weight just about covers the minimum requirement for young calves. At this level of feeding the calves grew fairly well although not to the same extent as those receiving larger amounts of vitamin A. There was no evidence of night blindness in

three of the four calves in this group and histological examination revealed none of the characteristic changes of vitamin A deficiency, namely, metamorphosis of the epithelium into keratinizing stratified squamous epithelium. These observations on the minimum vitamin A requirement of calves are in agreement with those of Guilbert, Miller and Hughes ('37) who found that 6 to 8 μg. of vitamin A (24 to 32 U.S.P. units) per kg. per day represent the minimum requirements for cattle, swine and sheep.

TABLE 2

Relationship of vitamin A intake to liver storage.

VITAMIN A INTAKE PER KILOGRAM OF BODY WEIGHT	CALF NO.	VITAMIN A UNITS PER 100 GM. OF LIVER	VITAMIN A UNITS IN THE WHOLE LIVER
32	403	0	0
	415	640	17,433
	422	0	0
	425	1,600	31,088
64	401	150	6,129
	406	0	0
	424	0	0
128	410	280	15,890
	402	580	18,432
	419	620	11,959
	427	580	13,821
256	412	1,260	45,343
	423	1,000	47,670
	428	3,840	78,450
512	413	1,390	64,676
	408	2,620	95,158
	421	440	11,479
1,024	414	1,650	69,283
	407	12,600	457,632
	426	7,300	132,568
	430	210	4,546

The amount of vitamin A required for optional growth was 64 units per kilo whereas an intake of 512 units was necessary to bring about maximal concentrations of vitamin A in the blood plasma. Moderate storage of vitamin A in the liver resulted in those calves receiving 256 and 512 units per kilo and considerable amounts of vitamin A were obtained in the group receiving the highest intake of vitamin A, namely 1024 units.

Boyer and his co-workers ('42) report a vitamin A intake of 12 μg. per kilogram to be border line and 18 μg. as adequate in young calves.

Assuming that 1 μg. of vitamin A is equivalent to 4 U.S.P. units, the adequate level of 18 μg. corresponds quite well with the level of 64 units fed to group II which we found to be adequate for maximum growth. However, our results show that these intakes of vitamin A will not provide for maximal blood levels or significant storage in the liver.

From a practical standpoint it would seem desirable to feed calves a level of vitamin A which would allow for at least a moderate degree of storage. The results reported herein indicate an intake of 250 U.S.P. units per kilogram or 11,000 units per 100 pounds of live weight is required for this purpose.

The observations in this investigation are of special interest when compared with those reported by Lewis and his co-workers ('39, '42) in their experiments on the vitamin A requirements in infants and in rats. They found that the minimum requirements in infants as judged by dark adaptation was of the same order of magnitude as in rats, namely, 20 to 35 units per kilogram body weight. At this intake the infants grew fairly well and appeared normal in every way. However, the vitamin A concentration in the blood, at this level of intake, was found to be abnormally low. Maximal blood concentrations were obtained in infants as in young rats when the vitamin A intake was approximately twenty-five times the minimum requirements. In their experiments on young rats a fair degree of storage in liver occurred when the feeding was twenty-five times the minimum requirement and good reserves were noted in the livers when the intake was fifty times the minimum requirements.

The data reported in the present communication indicate that in young calves maximal blood levels of vitamin A occurred with an intake of sixteen times the minimum requirement. The storage in the liver was moderate when the amount of vitamin A fed was eight to sixteen times the minimum requirement; large stores in the liver were noted when the intake was thirty-two times the minimum requirement. Thus, in general, the same situation occurs in calves as in rats and in infants with respect to the relationship of vitamin A intake to growth, concentration of vitamin A in the blood and liver storage. Small amounts of vitamin A bring about good growth whereas considerably larger amounts are required for maximal blood levels and good liver storage.

In this connection it is of interest to mention that only one of four calves receiving the low intake of 32 units per kilogram body weight exhibited night blindness. We have previously demonstrated that the retinal requirement for vitamin A is very low and that with the ingestion of small amounts of vitamin A by rats, maximal concentrations of

vitamin A were obtained in the retinas even though the blood levels were low and the liver reserves were nil. It was therefore not surprising to find that three of the four calves receiving 32 units of vitamin A had normal dark adaptation despite the fact that the vitamin A concentration in the blood was very low. From a practical point of view, the blood level of vitamin A is a more sensitive indicator of the vitamin A status of the organism than is dark adaptation, as the latter becomes impaired only when the deficiency is of a more advanced degree.

SUMMARY AND CONCLUSIONS

Six groups of four calves each were fed various levels of vitamin A, ranging from 32 to 1024 U.S.P. units per kilogram of body weight per day. Data were obtained on rate of growth, blood levels of vitamin A and liver storage.

The results indicate that 32 units per kilogram of body weight just about cover the minimum requirement. At this level the calves grew fairly well and in most instances showed no evidences of night blindness. However, the blood concentration of vitamin A was low and there was little or no liver storage.

Maximum growth was obtained on an intake of 64 U.S.P. units per kilo of body weight.

The concentration of vitamin A in the blood was proportional to the intake until 512 units were given at which level maximal blood concentrations were obtained.

Liver storage of vitamin A was quite variable. The majority of the calves receiving 64 units per kilogram or below showed no liver storage. In general, liver stores were quite low for calves receiving 32, 64 and 128 units per kilogram, whereas moderate amounts of vitamin A were found in the livers of those fed 256 and 512 units and large amounts in the 1024 units group.

From the standpoint of both growth and liver storage the daily intake of vitamin A for young calves should be about 250 U.S.P. units per kilogram of body weight or 11,000 units per 100 pounds of liver weight.

The vitamin A requirements in calves are of the same order of magnitude as in young rats and in infants.

LITERATURE CITED

BOYER, P. D., P. H. PHILLIPS, N. S. LUNDQUIST, C. W. JENSEN AND I. W. RUPEL Vitamin A and carotene requirements for the maintenance of adequate blood plasma vitamin A in the dairy calves J. Dairy Sci., vol. 25, p. 433.

GUILBERT, H. R., R. F. MILLER AND E. H. HUGHES 1937 The minimum vitamin A and carotene requirement of cattle, sheep and swine. J. Nutrition, vol. 13, p. 543.

LEWIS, J. M., AND CHARLES HAIG 1939 Vitamin A requirements in infancy as determined by dark adaptation. J. Pediat., vol. 15, p. 812.

LEWIS, J. M., O. BODANSKY, K. G. FALK AND G. MCGUIRE 1942 Vitamin A requirements in the rat. J. Nutrition, vol. 23, p. 351.

THE INFLUENCE OF ASCORBIC ACID ON THE ACTIVITY OF GONADOTROPIC HORMONES IN GUINEA PIGS[1]

J. T. REID AND J. F. SYKES

Departments of Dairy and Physiology and the Institute of Nutrition, Michigan State College, East Lansing

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Recent evidence has been presented which indicates that vitamin C is related to normal reproductive function.

In their studies on vitamin C-gonadotropic hormone relationships, Erb and Andrews ('42) found a 42 to 67% reduction in the plasma ascorbic acid concentration of dairy bulls within 24 hours after the injection of 2250 R. U. of gonadotropin. They also reported a 20 to 50% reduction in plasma vitamin C level of cows during the first 24-hour period following the injection of 1000 to 2250 R. U. of gonadotropic hormone. Further study by Andrews and Erb ('42) using the castrate bovine showed that gonadotropic hormone may act independently of the gonads in effecting a reduction of the plasma vitamin C level. These results indicated that vitamin C was in some manner concerned with the utilization of gonadotropic hormones.

Giedosz (cited by Di Cio and Schteingart, '42) however, showed that in rats the simultaneous injection with 50 mg. of vitamin C and 25 R. U. of pregnant mare serum gonadotropin did not effect a greater increase in the weight of testes, ovaries or uterus than did the injection of 25 R. U. of gonadotropin alone. In a further study Andrews and Erb ('43) found no relationship between the plasma ascorbic acid level and testes weight in young chicks, and concluded that the effects of gonadotropic hormone are not augmented by vitamin C.

Giedosz (cited by Di Cio and Schteingart, '42) however, showed that larger ovaries are produced in adult rabbits injected simultaneously with vitamin C and gonadotropin for 3 days than were produced in animals injected with hormone alone. He concluded that the hormone is more efficient when vitamin C saturation is accomplished. These findings were similar to those of Di Cio and Schteingart ('42) who used 2 to 4 months old white rats. While the latter investigators showed no

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appreciable increase in gonadal size of rats administered C alone over the normals, a much greater increase in the weight of gonads was found in those rats simultaneously injected with 50 mg. of vitamin C and 25 R. U. of gonadotropin than in those receiving gonadotropic hormone alone.

It seems possible to assume that the failure of Andrews et al. to potentiate the effects of gonadotropins with vitamin C in the rat and chicken was due to the fact that these animals are able to synthesize the vitamin. It was the object of this study to determine whether or not vitamin C increases the effects of gonadotropic hormone in the guinea pig since this animal does not synthesize the vitamin, but depends on a food source to supply its needs.

MATERIALS AND METHODS

Data on fifty-eight male and sixty-one female guinea pigs composing five groups were obtained in this study.

All groups received the basal, low vitamin C diet with certain modifications which will be discussed under each individual group. The basal ration had the following composition (parts by weight): skim milk (dried) 30, rolled oats 30, bran 30, butterfat 10, NaCl 1, and cod liver oil 1. The cod liver oil contained 400 and 3000 units per gram of vitamins D and A, respectively.

Group 1 received the low vitamin C diet throughout the experiment without supplementary vitamin C or gonadotropin. Group 2 received the basal diet and gonadotropin but did not receive supplementary ascorbic acid. After the appearance of scorbutic symptoms, gonadotropin was injected at two levels. One hundred R. U. and 50 R. U. of gonadotropin was injected daily into seventeen and twenty-five guinea pigs, respectively, for 4 days. Group 3 was depleted of vitamin C on the basal diet until deficiency symptoms were evident; and, then given 50 mg. of vitamin C daily for 3 days, followed by a 4-day period during which gonadotropin in addition to 50 mg. of ascorbic acid was administered. Twelve animals were injected with 100 R. U. of gonadotropin per day while the remaining twenty were injected with 50 R. U. per day. Group 4 received the basal diet supplemented with 2 mg. of ascorbic acid per guinea pig per day. When scorbutic symptoms were manifested in the animals of groups 1, 2 and 3, daily injections of 100 R. U. and 50 R. U. were administered, respectively, to eight and nine animals of group 4 for 4 days. Group 5 received the basal diet and 2 mg. of ascorbic acid per guinea pig per day until 9 days prior to the termination of the experiment when the daily diet for each animal was supplemented with 50

mg. of ascorbic acid. Six animals of this group were injected with 100 units of gonadotropin for 4 days while the remaining nine guinea pigs were injected with 50 units per animal for the same period of time.

In all cases where gonadotropin injections were given, the hormone was injected for 4 days and the animals were killed on the sixth day after the initial injection. The gonads were trimmed free of extraneous tissue, except for the epididymis of the testes, and weighed immediately. Gonadin serum from pregnant mares containing 50 R. U. per milliliter was used for the injections indicated. Supplementary vitamin C for the groups indicated above was mixed in the feed daily previous to feeding. The irritation effects which usually accompany injection of the vitamin were thus eliminated.

Since several workers, Rosenberg ('42), Farmer and Abt ('36) and Eddy and Dalldorf ('44), have indicated that the plasma vitamin C level reflects the degree of deficiency, this test as well as scorbutic symptoms was used as a criterion of the degree of deficiency. The vitamin C level was determined on the blood plasma of representative animals from each group. Care was taken in the handling of blood drawn from the heart for plasma ascorbic acid determinations to avoid exposure to light and heat. Although the general procedure outlined by Mindlin and Butler ('37) for the determination of vitamin C was employed, a modification (unpublished data) was introduced to preclude error due to a visually undetectable turbidity of the filtrates. This modification consisted of a complete reduction of the 2–6 dichlorophenol indophenol dye after reduction of the dye by the blank and unknown had been determined. A correction was then made for each on the basis that the completely reduced solution of dye and filtrate gave 100% transmission of light when the instrument was adjusted for 100% transmission with distilled water. Readings were made at 520 μ on a Cenco spectrophotelometer equipped with a Rubicon galvanometer.

RESULTS

The data in table 1 show the male and female gonad weights and the initial and terminal plasma vitamin C levels for groups 1, 2 and 3. Inasmuch as no difference between the effects of 100 or 50 units of gonadin was observed, the gonad weights for these animals have been grouped together.

The figures show that the average testes weight for the animals in groups 2 and 3 were, respectively, 1.81 and 1.83 times greater than those in group 1, whereas the average testes weight for group 3 was only 1.0094 times heavier than the male gonads in group 2.

The respective average ovary weights for groups 2 and 3 were 1.73 and 2.37 times as great as those in group 1, and the mean ovary weight for the animals of group 3 was 1.38 times as heavy as those in group 2. The differences observed between groups 1 and 2 may not be significant due to the small number of animals which survived in group 1. These data, however are an indication that gonadotropins are effective in the vitamin C deficient guinea pig and group 1 was included in the experimental set-up for this purpose.

TABLE 1

The effect of the low vitamin C diet, gonadotropin alone, and the combination of vitamin C and gonadotropin on gonad weight.

GROUP	SUPPLEMENT		MALES [1]	FEMALES [1]	PLASMA VITAMIN C [2]		TERMINAL BODY WEIGHT
	Vitamin C	Gonadotropin	Mean gonad weight	Mean gonad weight	Initial	Terminal	
	mg.	*R.U.*	*gm.*	*mg.*	*mg. %*	*mg. %*	*gm.*
1	0	0	1.354 ± 0.20(10)	51 ± 8.15(3)[3]	0.258(6)	0.019(6)	326
2	0	50 or 100 (4 days)	2.455 ± 0.18(19)	88 ± 3.47(23)	0.318(18)	0.077(10)	312
3	50 (7 days)	50 or 100 (4 days)	2.478 ± 0.19(15)	121 ± 7.42(17)	0.304(18)	0.517(15)	371

[1] Figures in parentheses indicate number of animals or determinations.

[2] Average values.

[3] The small number of females in group 1 is actually the number that existed when the experiment terminated, and is small because of a high rate of mortality due to scurvy. Originally the number of animals in groups 1, 2 and 3 was comparable. Although the direction of the results was the same for each of the three females in group 1, the chief comparison to be stressed here is based on groups 2 and 3, i.e., demonstrating the potentiation of the effects of gonadotropins by vitamin C.

Although the supplementation of the diet of male animals with ascorbic acid did not potentiate the effects of gonadotropin, these data indicate a marked synergistic relationship involving the plasma vitamin C concentration and gonadotropic hormone in female guinea pigs as indicated by the increased gonad weights of the females in group 3 as compared to group 2.

The weight of the gonads and the initial and terminal plasma vitamin C values for groups 4 and 5 are shown in table 2. A comparison of these data with those for group 2 in table 1 shows that the average ovary weight of guinea pigs which were normal with respect to plasma ascorbic acid concentration and which were injected with gonadotropin was greater than in similar animals receiving the basal diet and gonadotropic hormone alone. The addition of 50 mg. of vitamin C daily for 9

days to normal guinea pigs did not increase the effectiveness of gonadotropins.

It will be noted that the terminal ascorbic acid values for group 4 are lower than the initial values and that in group 5 the addition of extra vitamin C did not produce terminal values comparable to those obtained in group 3. These, however, are average values and the lower terminal values are a result of the fact that 2 mg. of vitamin C per day in the ration failed to maintain the plasma ascorbic acid at comparable levels in all animals in these two groups.

TABLE 2

Effect of gonadotropin on guinea pigs with normal plasma vitamin C concentration.

GROUP	SUPPLEMENT		MALES [1]	FEMALES [1]	PLASMA VITAMIN C [2]		TERMINAL BODY WEIGHT
	Vitamin C	Gonadotropin	Mean gonad weight	Mean gonad weight	Initial	Terminal	
	mg.	*R.U.*	*gm.*	*mg.*	*mg. %*	*mg. %*	*gm.*
4	2 (daily)	50 or 100 (4 days)	2.134 ± 0.29(10)	134 ± 13.19(7)	0.234(9)	0.127(6)	420
5	2 (daily); 50 (9 days)	50 or 100 (4 days)	2.170 ± 0.57(4)	117 ± 7.02(11)	0.228(8)	0.326(6)	382

[1] Figures in parentheses indicate number of animals or determinations.

[2] Average values.

DISCUSSION

Inasmuch as the failure of other investigators to show an augmentation of the effects of gonadotropin by ascorbic acid may have been due to the use of experimental animals capable of ascorbic acid synthesis, the guinea pig was chosen as the test animal for this study by virtue of its inability to synthesize vitamin C. It seemed logical that the effects of the vitamin on gonadotropin utilization could be interpreted more conclusively in the guinea pig since this animal may be readily depleted or maintained at various vitamin C levels. In the first three groups receiving the low vitamin C diet, scorbutic symptoms accompanying a decreased plasma vitamin C concentration were manifested after a period of 3 to 4 weeks. Although a great response to the supplementary vitamin C was demonstrated by depleted females injected with gonadotropin, no noticeable potentiation was found in the males. This may be explained partially by a greater resistance to vitamin C depletion as evidenced by lack of marked scorbutic symptoms in the males as compared to the females.

The augmentation effects of ascorbic acid observed in female guinea pigs in this study offers support to the results obtained with rabbits and rats reported by Giedosz (cited by Di Cio and Schteingart, '42) and Di Cio and Schteingart ('42), respectively. The failure of Andrews and Erb ('43) to show the potentiation of gonadotropin effects by ascorbic acid in male chicks may be attributed to a sex as well as a species difference relative to the ascorbic acid-gonadotropin synergism. Almquist and Andrews ('43) also failed to duplicate the results of Di Cio and Schteingart ('42) with rats. It is difficult to offer an explanation for this discrepancy in results when the latter species was used.

The augmentation of the effects and the utilization of gonadotropic hormone produced by vitamin C appear to vary with sex as well as species. The difficulty commonly encountered in the treatment of sterility in various animals with gonadotropins might be attributed in part to this fact.

SUMMARY

Gonadotropin injections in vitamin C depleted females resulted in an average ovary weight 1.73 times greater than in depleted females not receiving gonadotropin injections. The addition of vitamin C further increased the gonadotropic effects and produced ovaries 1.38 times heavier than in those receiving gonadotropin alone.

The injections of 50 or 100 R.U. of gonadotropin into vitamin C depleted male guinea pigs increased the testis weight 1.81 times as compared to the depleted guinea pigs not receiving gonadotropin. Supplementing the basal diet with 50 mg. of vitamin C per day, however, did not further increase the testis weight produced by gonadotropin alone.

The augmentation of the effects and the utilization of gonadotropic hormone caused by vitamin C appears to differ with sex as well as species.

LITERATURE CITED

ALMQUIST, J. O., AND F. N. ANDREWS 1943 Ascorbic acid-gonadotropic hormone relationships. J. Animal Sci., vol. 2, p. 356.

ANDREWS, F. N., AND R. E. ERB 1942 Effect of the gonadotropic substance of pregnant mare's serum on the blood plasma-ascorbic acid of the castrate bovine. Endocrinol., vol. 30, p. 671.

———— 1943 Ascorbic acid-gonadotropic hormone relationships in the chick. Endocrinol., vol. 32, p. 140.

DI CIO, A. V., AND M. SCHTEINGART 1942 The influence of ascorbic acid on the activity of gonadotropic hormones. Endocrinol., vol. 30, p. 263.

EDDY, W. H., AND G. DALLDORF 1944 The Avitaminoses. Williams and Wilkins Company, Baltimore, 3rd ed., pp. 294–296.

ERB, R. E., AND F. N. ANDREWS 1942 Effect of the gonadotropic substance of pregnant mare's serum on the blood plasma-ascorbic acid of the bovine. Endocrinol., vol. 30, p. 258.

FARMER, C. J., AND A. F. ABT 1936 Determination of reduced ascorbic acid in small amounts of blood Proc. Soc. Exp. Biol. Med., vol. 34, p. 146.

MINDLIN, R. L., AND A. M. BUTLER 1937 The determination of ascorbic acid in plasma; a macromethod and micromethod. J. Biol. Chem., vol. 122, p. 673.

ROSENBERG, H. R. 1942 Chemistry and Physiology of the Vitamins. Interscience Publishers, Inc., New York, p. 333.

THE AVAILABILITY OF THE IRON IN HAWAIIAN-GROWN VEGETABLES [1]

CAREY D. MILLER AND LUCILLE LOUIS [2]
Nutrition Department, Hawaii Agricultural Experiment Station, University of Hawaii, Honolulu, T. H.

ONE FIGURE

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In Hawaii there has been for some years a popular belief that locally produced vegetables may be low in minerals, notably iron, and that people residing in the Islands tend to become anemic. These erroneous ideas probably arose because it is generally known that pineapples grown in certain areas become chlorotic and must be sprayed with iron sulfate. Johnson ('24) showed that precipitation of the iron by manganese in certain soils deprives the plant of the iron required for chlorophyll synthesis despite the presence of abundant iron. Later work (Sideris, Young and Krauss, '43) has proved that not only manganese, but calcium, phosphorus and the pH of the soil play important roles in the utilization of iron by the pineapple plant. Other plants growing in the same soil with chlorotic pineapple plants do not show chlorosis.

Blood studies (Hamre, '39) on local residents have shown the usual range of hemoglobin and red cell counts found on the mainland. Analyses of a large number of locally produced vegetables for calcium, phosphorus and iron (to be published elsewhere) have shown no greater range in values than reported for other regions.

This study reports the results of iron analyses of fifteen vegetables and two seaweeds grown in Hawaii and the availability of the iron for the regeneration of hemoglobin in anemic rats.

EXPERIMENTAL

Rats. Young rats were made anemic by a modification of the Elvehjem and Kemmerer ('31) technique. The mothers were fed the stock diet throughout the lactation period; but from the time the young were

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14 days old, the mothers were removed from the cages for feeding twice a day and their coats brushed before returning them to the cages with the young. At 17 days of age the young and the mothers had access to whole powdered milk. The young were weaned at 21 days, placed in individual, new, rust-free, galvanized cages, and fed an exclusive diet of whole milk powder and tap water.

As Smith and Otis ('37) had reported a greater hemoglobin response for females than males when fed equivalent amounts of iron, only male rats were used in our experiments. Weekly blood samples were taken from the rats' tails by snipping off a bit of the tip or by cutting a small gash near the end of the tail with a sharp razor. Care was taken to obtain a full-flowing sample and to stop the bleeding promptly after the samples were taken. The average period for depletion of hemoglobin stores was 26 days. Most of the rats were depleted to a level of about 3.4 gm. of hemoglobin per 100 ml. of blood in 21 to 24 days but two litters for undetermined reasons took 30 and 40 days.

To determine a curve of response, one hundred male rats were divided into nine groups, with proper distribution of the rats from different litters to each group, and, after depletion of hemoglobin, were fed daily (except Sunday) supplements of iron (ferric chloride) for a period of 6 weeks as follows: 0.015, 0.03, 0.06, 0.10, 0.13, 0.17, 0.20 and 0.25 mg. Each rat was also fed daily supplements of 0.05 mg. copper in the form of a dilute solution of $CuSO_4$ and 0.04 mg. manganese in the form of a dilute $MnSO_4$ solution. One group of eleven rats received these supplements without iron. Statistical analysis of the data on gains in hemoglobin showed that feeding the supplements for 4 weeks produced as significant results as for 5 or 6 weeks, somewhat better than 3 weeks and definitely better than 2 weeks. The minimum number of rats necessary to give a significant result in hemoglobin response after 4 weeks of feeding was calculated to be nine for the procedure and the rat colony used. A linear regression curve was calculated from the data for the 4-week feeding period and used as a curve of reference for the experiments which followed (fig. 1). Complete data for all groups of rats used for the curve of response have been omitted from series 1, table 1, in order to save space and only the data for the groups fed supplements of copper and manganese without iron, 0.06 mg. of iron, and taro leaves are included.

For the final tests of the available iron in foods, two groups of rats in each series received 0.06 and 0.08 mg. of iron. All groups received the copper and manganese supplements. The plan was to feed all supplements for a period of 4 weeks. It was necessary to modify the plan

of the feeding experiments when, in March, 1942, it was obvious that our supplies of powdered whole milk were running low and all stores of such foods in Hawaii were being reserved for human consumption. There were, moreover, shortages in staff and the uncertainty as to how long research work could continue. Consequently, in order to complete the project and utilize the rats on the experiment, the period for feeding the supplements for series 5 and 6 was reduced to 3 weeks (see fig. 1 for linear regression curve) and for series 6 only one group was fed 0.06 mg. of iron as a positive control.

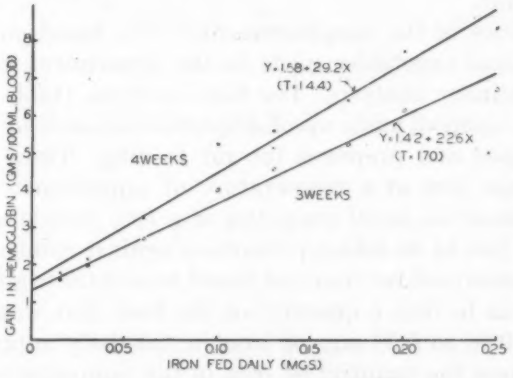

Fig. 1 Curves of response (linear regression curves for gains in grams of hemoglobin per 100 ml. of blood in relation to milligrams of iron fed daily, with supplements of copper and manganese, for 3- and 4-week periods).

Supplements and their preparation. All of the vegetables were fed in the fresh or cooked state as it was thought that drying and grinding might adversely affect the utilization of the iron. The limu (Hawaiian name for all seaweeds), gathered from the seashore or rocks, were thoroughly cleaned and washed and fed in the raw state. The vegetables were prepared as for human consumption, discarding such portions as old leaves, over mature beans, or lower tough stalks of asparagus. In the case of the white mustard cabbage, a portion of the large white petiole was discarded in order that a smaller sample with a higher iron content might be used for feeding purposes. (The entire edible portion of the plant contained 0.00053% of iron by one set of analyses, whereas the composite sample used for rat feeding contained 0.00156% of iron.)

The vegetables were cooked in aluminum vessels and chopped on a rubber chopping board with a stainless steel knife. Samples were prepared once or twice a week and stored in glass jars in the refrigerator until fed.

The green leafy vegetables, green beans, and cowpeas were steamed for 10 to 15 minutes, without loss of liquid. The green soy beans were cooked in boiling water in the pods for 20 minutes; the beans were shelled from the pods and chopped before feeding. Other vegetables — carrots, asparagus, green lima beans (shelled) — were cooked for varying lengths of time until tender, without loss of liquid. The cooked, ground taro was a commercial product locally known as paiai, containing about 30% solids. It was put through a coarse cloth (poi cloth) without the addition of water to remove the lumps and to make a more uniform sample.

The quantities of the supplements fed were based on previous iron analyses of local vegetables made in the department laboratory or a special preliminary analysis. The final analyses (table 1) were made on composite samples made up of aliquots from each lot of vegetables, cooked, chopped and prepared for rat feeding. These samples were stored in glass jars at a temperature of approximately 34°F. until analyzed. Measured, small quantities of a 10% formalin solution were added to the jars as an added precaution against molds. The formalin solution was analyzed for iron and found to contain negligible amounts.

The aim was to feed a quantity of the food that would furnish approximately 0.06 to 0.08 mg. of iron in the daily supplements. In a number of cases the quantity of iron in the composite sample, proved to be either higher or lower than that found by preliminary analyses, so that the amount of iron furnished in the sample fed proved to be smaller (limu lipeepee) or larger (white mustard and green mustard cabbage) than was calculated.

Iron analyses. In preparing the samples for analyses, every precaution was taken to guard against contamination with iron from outside sources. The samples were ashed without drying. For each sample analyzed, two checks were run for the entire process from ashing to colorimetric readings — one a complete blank, the other with a known quantity of added iron. The Saywell and Cunningham ('37) method of color development with ortho-phenanthroline was followed. A Klett-Summerson photoelectric colorimeter was used for reading the solutions.

The iron solutions for feeding were made from either special reagent ferric chloride or pure iron wire, and the iron contents were checked by the ortho-phenanthroline method. The copper and manganese salts were tested for their iron contents and found to have only a trace — too small an amount to detect in the dilute solutions used.

Hemoglobin determinations. Hemoglobin was determined by the acid hematin method, using a Klett-Summerson photoelectric colori-

Summary of the results of feeding various Hawaiian foods and ferric chloride as sources of iron to anemic rats.

SUPPLEMENTS FED DAILY (EXCEPT SUNDAYS)	IRON IN FOOD		NO. OF RATS	HEMOGLOBIN			APPROXIMATE % OF Fe UTILIZED	AVERAGE WEIGHTS		
				When started	4th week			Initial	At depletion	End of expt.
	mg./ 100 gm.	Furnished daily			Level	Gain				
		mg.		*gm./100 ml.*	*gm./100 ml.*	*gm./100 ml.*	%	*gm.*	*gm.*	*gm.*
Series 1.										
$CuSO_4$(0.05 mg. Cu) + $MnSO_4$(0.04 mg. Mn)		0	11	3.3	4.5	1.2		42	93	146
Ferric chloride		0.06	11	3.2	6.5	3.3		41	90	180
Taro leaves 5 gm.	1.42	0.071	10	3.4	6.9	3.5	93	45	104	196
Series 2.										
Ferric chloride		0.06	10	3.4	7.2	3.8		43	98	183
Ferric chloride		0.08	10	3.5	7.7	4.2		43	96	187
Cowpeas (fresh pods) 8 gm.	1.12	0.09	10	3.4	7.0	3.6	65	44	95	195
Swamp cabbage 5 gm.	2.62	0.131	10	3.4	7.7	4.3	59	44	98	183
Green beans 10 gm.	0.78	0.078	10	3.5	6.4	2.9	49	44	99	181
Series 3.										
Ferric chloride		0.06	10	3.3	6.6	3.3		39	87	178
Ferric chloride		0.08	10	3.3	7.3	4.0		39	88	180
Amaranth 5 gm.	2.97	0.149	10	3.3	6.2	2.9	32	40	87	177
Lima beans (green) 4 gm.	1.83	0.073	10	3.2	6.3	3.1	74	39	80	185
Watercress 7 gm.	1.37	0.096	10	3.2	5.6	2.4	31	38	81	174
Series 4.										
Ferric chloride		0.06	10	3.6	7.0	3.4		41	93	193
Ferric chloride		0.08	10	3.7	7.3	3.6		42	97	193
White mustard cabbage 9 gm.	1.56	0.14	10	3.6	6.5	2.9	34	41	95	200
Green mustard cabbage 9 gm.	1.21	0.109	10	3.6	5.4	1.8	10	40	95	179
Paiai (taro corm) 7 gm.	1.52	0.106	10	3.6	7.9	4.3	93	40	98	200
Series 5.					Third week					
Ferric chloride		0.06	10	3.6	6.1	2.5		43	99	166
Ferric chloride		0.08	10	3.7	6.7	3.0		44	100	171
Asparagus 10 gm.	1.82	0.182	10	3.7	5.3	1.6	6	42	98	163
Broccoli 10 gm.	1.02	0.102	10	3.6	6.0	2.4	52	43	100	180
Carrots 10 gm.	0.47	0.047	10	3.6	5.1	1.5	17	42	100	171
Series 6.										
Ferric chloride		0.06	10	3.7	6.5	2.8		44	105	165
Green soybeans 3.5 gm.	2.98	0.104	10	3.6	7.4	3.8	96	44	105	182
Limu lipoa 1.0 gm.	15.02	0.15	10	3.6	7.2	3.6	62	44	106	171
Limu lipeepee 0.2 gm.	21.40	0.043	10	3.6	4.6	1.0		41	103	166
Belembe 6.0 gm.	2.73	0.164	10	3.6	6.2	2.6	31	44	106	160

meter for measurement of the concentration of the solutions. The readings of the colorimeter were standardized with a series of acid hematin solutions made from a sample of blood of which the iron content was determined by the Wong ('28) method.

"Available" or acid-soluble iron. Many unsuccessful attempts to determine available iron were made in this laboratory using the alpha alpha-dipyridyl method and its numerous modifications (Shackleton and McCance, '36; Kohler, Elvehjem and Hart, '36; Ranganathan, '38.) We obtained more consistent results if the acetic acid solutions were evaporated and ashed and the color developed with ortho-phenanthroline than if dipyridyl was used for development of color directly in the extracts. For all but one of the foods studied, acid extracts were also made with 7.5% sulphuric acid.

Briefly the methods used were as follows: Raw or cooked food samples were ground in a special aluminum grinder. For the acetic acid extracts, 40 to 50 ml. of 10% acetic acid were added to 20 to 25 gm. of finely ground samples and the mixtures heated in beakers on the waterbath for 2 to 3 hours. After adding 1 ml. of 10% hydroxylamine hydrochloride, the heating was continued for another hour. After standing overnight, the samples were transferred to 50 ml. tubes and centrifuged. The supernatant liquid was filtered off and the residue was washed four times with 4% acetic acid followed by centrifuging. The solutions were transferred to silica dishes and after evaporation on a waterbath residues were ashed as for total iron with the usual precautions and customary blanks.

For the sulphuric acid extracts, 20 to 30 ml. of 7.5% sulphuric acid were added to samples weighing 10 to 15 gm. The mixtures were heated on the waterbath and centrifuged in the same way as for the acetic acid extractions. After evaporating nearly to dryness, digestion was completed by the addition of nitric and perchloric acids. Solutions were made to volume and iron determined as previously described.

RESULTS AND DISCUSSION

The Hawaiian-grown foods studied, their total iron contents, and their hemoglobin regenerating values as determined by bioassay are summarized in table 1.

The values for total iron in six of the vegetables studied were compared with values in the literature that were obtained when special precautions were taken to guard against iron contamination (Stiebling, '32; Peterson and Elvehjem, '28). Three of the Hawaiian-grown vegetables — asparagus, green snap beans and lima beans — had as much iron

as the mainland products, and two — broccoli and carrots — had slightly less iron. The Hawaiian-grown watercress (composed of 3 to 4 inches of stems plus leaves) contained much less iron than was reported by these two laboratories. However, our results compare favorably with those reported for the iron content of cultivated watercress from West Virginia (Stegner, '44). The green soybeans used contained as much iron as those grown in Illinois (Woodruff and Klaas, '38). Reliable iron figures for the total iron content of the other vegetables studied could not be found in the literature.

The percentages of "available iron" determined by the two acid extraction methods were in most cases very divergent and neither of them showed any correlation with the results by bioassay. The data are summarized in table 2.

TABLE 2

"Availability" of iron. (Expressed as percentage of total iron.)

FOOD	BIOASSAY	SOLUBLE IN 10% ACETIC ACID	SOLUBLE IN 7.5% SULPHURIC ACID	FOOD	BIOASSAY	SOLUBLE IN 10% ACETIC ACID	SOLUBLE IN 7.5% SULPHURIC ACID
	%	%	%		%	%	%
Amaranth	32	14	99	Cabbage			
Asparagus	6	26	86	green mustard	10	47	74
Beans				swamp	59	4	69
lima (green)	74	10	101	white mustard	34	33	103
green snap	49	31	95	Limu lipoa (seaweed)	62	23	99
green soy	96	12	(113)	Paiai (taro corm)	93	49	92
Belembe	31	5	92	Taro leaves	93	4	..
Broccoli	52	29	99	Watercress	31	21	81
Cowpeas (pods)	65	13	99				
Carrots	17	45	(115)				

On the basis of bioassays with anemic rats, nine of the foods studied had 50% or more of the iron in available form and seven had less than 50%. In agreement with other published reports (Sheets and Ward, '40; Theriault and Fellers, '42), we found fresh legume seeds to have a relatively high percentage of available iron — soybeans 96% and fresh lima beans 74%. The green podded legumes with seeds — cowpeas and green snap beans — yielded lower values, 65 and 49%, respectively.

The iron in taro corms (paiai) and taro leaves was found to be 93% available. Both foods are important items in the diet of ancient and modern Polynesians.

Others have reported the iron of green leafy vegetables to be generally less available than the iron in legumes (Sheets and Ward, '40; Theriault and Fellers, '42). We found the available iron by bioassay to vary greatly — from 93% in taro leaves to 10% in green mustard cabbage. Two leafy vegetables, taro leaves and swamp cabbage, had more than 50% of the iron available, four — white mustard cabbage, amaranth, watercress and belembe — had about 30% available and one, green mustard cabbage, had only 10% available.

We found broccoli to have only 52% of its iron available by bioassay, whereas Hastings, Fellers and Fitzgerald ('41) reported 71% for raw broccoli and 94% for fresh frozen broccoli. Fresh, cooked, Hawaiian-grown asparagus yielded the lowest value for available iron of all the vegetables studied — 6%. Theriault and Fellers ('42) report 55% available iron bioassay for quick-frozen asparagus fed in dried form.

Insufficient iron was furnished by one of the seaweeds (limu lipeepee, a species of Laurencia) to make an estimate of its availability. The quantity fed was based on a preliminary iron analysis of a sample that was probably not fully freed from sand. The data in table 1 for the other seaweed, limu lipoa, indicate that the iron is relatively well utilized.

On the basis of these experiments, eight vegetables and one seaweed appear to be good sources of iron for hemoglobin building. A number of the green leafy vegetables commonly considered good sources of iron were found to be relatively poor for hemoglobin regeneration in the anemic rat.

The large gains in weight (table 1) made by our rats during the 3- or 4-week period when either inorganic iron or the food supplements were added to the milk powder diet appear to be greater than are reported by other investigators (Harris, Mosher and Bunker, '39; Nakamura and Mitchell, '43). A portion of the iron ingested was undoubtedly used for purposes other than blood hemoglobin as Hahn et al. ('39) have pointed out that iron exists in many places in the body other than red cell hemoglobin. If growth had been retarded by restriction of the basal diet of milk powder, probably the increases in hemoglobin would have been greater, but this should not have influenced the relationship of the increases between the groups fed food supplements and the controls.

Sherman ('41) has questioned whether the availability of iron of foods based on studies with anemic rats has any significance for humans. However, there would seem to be just as much justification for accepting the comparative values for the utilization of iron in foods as for accepting the comparative vitamin values of foods (especially vitamin A and carotene) and biological values of proteins determined by means of

rat experiments. All such values are relative and merely guides in planning human dietaries. Moreover, since Hahn et al. ('39) have shown by means of radioactive iron studies that the anemic animal absorbs iron more readily than the nonanemic one, it would appear unlikely that the iron of foods poorly used by anemic rats would be better utilized by normal humans.

SUMMARY AND CONCLUSIONS

The availability of the iron in fifteen Hawaiian-grown vegetables and one seaweed was determined by bioassay.

The mean values for hemoglobin regeneration in groups of anemic rats fed varying amounts of the foods for periods of 3 or 4 weeks were compared with those of control groups fed pure ferric chloride. All rats were given supplements of copper and manganese.

In comparison with the utilization of the iron in ferric chloride, the percentages of the total iron available in the foods studied were found to be as follows: green soybeans 96, taro corms 93, taro leaves 93, fresh lima beans 74, cowpeas (pods) 65, a seaweed, limu lipoa (Haliseris plagiogramma) 62, swamp cabbage (Ipomoea aquatica) 59, broccoli 52, green snap beans 49, white mustard cabbage (Brassica chinensis) 34, amaranth (Amaranthus gangeticus) 32, watercress 31, belembe (Xathosoma brasiliense) 31, carrots 17, green mustard cabbage (Brassica juncea) 10, and asparagus 6.

The superior quality of the iron of legume seeds for hemoglobin regeneration in the rat was confirmed. Taro corms and taro leaves, both important items in the diet of the Polynesians, were shown to have a high percentage of available iron.

The percentage of the food iron soluble in dilute acetic or in dilute sulphuric acid bore no relationship to the availability as determined by bioassay.

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LITERATURE CITED

ELVEHJEM, C. A., AND A. R. KEMMERER 1931 An improved technique for the production of nutritional anemia in rats. J. Biol. Chem., vol. 93, p. 189.

HAHN, P. F., W. F. BALE, E. O. LAWRENCE AND G. H. WHIPPLE 1939 Radioactive iron and its metabolism in anemia. J. Exp. Med., vol. 69, p. 739.

HAMRE, C. J. 1939 A blood survey of 137 young men and 112 young women. Trans. 49th Annual Meeting, Hawaii Terr. Med. Assn., p. 98.

HARRIS, R. S., L. M. MOSHER AND J. W. M. BUNKER 1939 The nutritional availability of iron in molasses. Am. J. Digest. Dis., vol. 6, p. 459.

HASTINGS, W. H., C. R. FELLERS AND G. A. FITZGERALD 1941 Effect of freezing on the available iron content of foods. Proc. Am. Inst. of Refrig., p. 1.

JOHNSON, M. O. 1924 Manganese chlorosis of pineapples; its cause and control. Hawaii Agr. Exp. Sta. Bull. 52.

KOHLER, G .O., C. A. ELVEHJEM AND E. B. HART 1936 Modifications of the bipyridine method for available iron. J. Biol. Chem., vol. 113, p. 49.

NAKAMURA, F. I., AND H. H. MITCHELL 1943 The utilization for hemoglobin regeneration of the iron in salts used in the enrichment of flour and bread. J. Nutrition, vol. 25, p. 39.

PETERSON, W. H., AND C. A. ELVEHJEM 1928 The iron content of plant and animal foods. J. Biol. Chem., vol. 78, p. 215.

RANGANATHAN, S. 1938 The "available" iron in some common Indian foodstuffs, determined by the alpha alpha-dipyridine method. Indian J. of Med. Res., vol. 25, p. 677.

SAYWELL, L. G., AND B. B. CUNNINGHAM 1937 Determination of iron — colorimetric *o*-phenanthroline method. Ind. Eng. Chem. Anal. Ed., vol. 9, p. 67.

SHACKLETON, L., AND R. A. MCCANCE 1936 The ionizable iron in foods. Biochem. J., vol. 30, p. 582.

SHEETS, O., AND M. V. WARD 1940 Studies in nutritional anemia. Miss. Agr. Exp. Sta. Tech. Bull. 26.

SHERMAN, H. C. 1941 Chemistry of Food and Nutrition. Macmillan, New York.

SIDERIS, C. P., H. Y. YOUNG AND B. H. KRAUSS 1943 Effect of iron on the growth and ash constituents of Ananas comosus (L.) Merr. Plant Physiol., vol. 18, p. 608.

SMITH, M. C., AND L. OTIS 1937 Hemoglobin regeneration in anemic rats in relation to iron intake. J. Nutrition, vol. 13, p. 573.

STEGNER, M. 1944 Home Ec. Consultant, New York. Personal communication.

STIEBLING, H. K. 1932 The iron content of vegetables and fruits. U.S.D.A. Cir. 205.

THERIAULT, F. R., AND C. R. FELLERS 1942 Effect of freezing and of canning in glass and in tin on available iron content of foods. Food Research, vol. 7, p. 503.

WONG, S. Y. 1928 Colorimetric determination of iron and hemoglobin in blood. J. Biol. Chem, vol. 77, p. 409.

WOODRUFF, S., AND H. KLASS 1938 A study of soybean varieties with reference to their use as food. Univ. Ill. Agr. Exp. Sta. Bull. 443.

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The formal presentation will be made at the annual meeting of the Institute in the spring of 1946. To be considered for the award, nominations must be in the hands of the Chairman of the Nominating Committee by January 15, 1946. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate consideration for the award.

W. E. KRAUSS
Ohio Agricultural Experiment Station
Wooster, Ohio

CHAIRMAN, NOMINATING COMMITTEE

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Nominations are solicited for the 1946 Award of $1,000 established by Mead Johnson and Company to promote researches dealing with the B complex vitamins. The recipient of this Award will be chosen by a Committee of Judges of the American Institute of Nutrition and the formal presentation will be made at the annual meeting of the Institute in the spring of 1946.

The Award will be given to the laboratory (non-clinical) or clinical research worker in the United States or Canada who, in the opinion of the judges, has published during the previous calendar year January 1st to December 31st the most meritorious scientific report dealing with the field of the 'B-complex' vitamins. While the award will be given primarily for publication of specific papers, the judges are given considerable latitude in the exercise of their function. If in their judgment circumstances and justice so dictate, it may be recommended that the prize be divided between two or more persons. It may also be recommended that the award be made to a worker for valuable contributions over an extended period but not necessarily representative of a given year. Membership in the American Institute of Nutrition is not a requisite of eligibility for the award.

To be considered by the Committee of Judges, nominations for this award for work published in 1945 must be in the hands of the Secretary by January 10, 1946. The nominations should be accompanied by such data relative to the nominee and his research as will facilitate the task of the Committee of Judges in its consideration of the nomination.

H. E. Carter
Noyes Laboratory of Chemistry
University of Illinois
Urbana, Illinois

Secretary, American Institute of Nutrition

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